Biofouling of salmon-cage netting: fouling quantification, removal and prevention

by

Stephen L. Hodson B.App.Sc. (Hons)

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Department of Aquaculture
University of Tasmania at Launceston

January, 1997

CONFIDENTIALITY

This thesis contains information that is commercially sensitive and confidential. No part of this thesis may be copied and it is not to be made available for loan for a period of two years from the date of submission without permission of the Aquaculture CRC Limited.

Stephen Hodson

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and to the best of my knowledge contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Stephen Hodson

Abstract

The quantification, removal and prevention of biofouling on salmon-cage netting were investigated in Tasmania, Australia. Trials were aimed at identifying environmentally-acceptable alternatives to traditional toxic antifoulants, and focussed on three areas, *viz.* (1) underwater cleaning, (2) a "foul-release" silicone coating, and (3) extruded polymers incorporating naturally-occurring compounds or a commercial short-lived biocide. Each of these strategies was found to be applicable and effective for the prevention and/or reduction of fouling on netting. Trials identified future directions to improve antifouling efficacy, and potential problems such as resistant organisms. The development of a quantification technique and the description of fouling in each trial also provided information on the composition and variability within fouling communities on netting.

Image analysis of close-up underwater photographs of net fouling was used to quantify fouling removal and regrowth in terms of mesh occlusion. This enabled large numbers of non-destructive samples to be taken and analysed quickly, which compensated for the high degree of natural variability in the fouling communities. It also provided a permanent record of the fouling, allowed dominant species to be identified and counted, and permitted multiple sampling of the same area.

Underwater cleaning of netting was found to be highly effective when there was good contact between the brushes and netting, and prevented significant fouling development over a 10 week period. However, the design of the trialed prototype cleaner and the displacement of netting away from the cleaner, reduced the severity of scrubbing, limiting fouling removal. This effectiveness was further compromised because residual structures of fouling organisms and the large quantity of debris led to rapid regrowth and recolonisation. The research identified areas for design improvement, the problems associated with residual fouling and regrowth, and the requirements for effective *in situ* net cleaning. The results demonstrate many difficulties that may limit the efficacy of *in situ* cleaning, but suggest that this technology can significantly extend the immersion time of nets during seasons of peak fouling.

The application of a commercial silicone coating (Viridian 2000, International Paints) to netting proved effective for increasing the ease of cleaning and reducing the total mass of attached fouling. The silicone coating was particularly effective at

preventing invertebrate fouling, and an absence solitary ascidians significantly reduced total fouling biomass. However, the total algal biomass on the silicone was comparable to uncoated netting, and some algal species were more abundant on the silicone (*Ulva rigida* and *Polysiphonia brodiaei*). This latter effect was considered to represent effects of surface colour, reduced competition, and differences in attachment strength. The application of current silicone coatings to fish-cage netting was considered to be problematic because their poor abrasion resistance and tear strength make them unsuitable for shore-based cleaning and handling procedures. Nonetheless, the flexibility of silicone coatings and the effective reduction of fouling adhesion make them ideal for fish cages that will be cleaned *in situ*, and may provide an effective non-toxic solution to reduce the cost of fouling removal.

Extruded polymers (plastics) incorporating either naturally-occurring compounds or commercially-available biocides were found to prevent macrofouling development for up to 209 days in field trials. The efficacy of polymers that incorporate either an algal extract (*Delisea pulchra* or *Laurencia rigida*), an analog to a halogenated furanone (that occurs in *D. pulchra*), or one of four commercial biocides (Busan 11-M1, Irgarol 1051, Nopcocide N-96 and Sea-nine 211) was investigated. Fouling prevention was shown to be a function of the polymer type and initial loading of the antifouling compound. Sea-nine 211 and the natural product analog were highly effective when incorporated into ethylene-vinyl acetate copolymers, and therefore this type of polymer may be suitable for mixtures of these antifouling compounds. A high-density polyethylene (Shell, HET 6100) and a copolymer of ethylene and acrylic acid (BASF, Lucalen® A) were also effective for Sea-nine 211. The current success with polymers that incorporate antifouling compounds, and the many options for increasing their efficacy, are encouraging for the development of netting which resists fouling.

Acknowledgements

I sincerely thank my supervisor Dr. Chris Burke (University of Tasmania) for his guidance, encouragement and brutal honesty throughout my candidature. I am grateful to Tom Lewis (University of Tasmania) for his ingenuity in operation of *in situ* cleaning equipment, even under conditions of near hypothermia, high seas and the occasional perplexing cryptic crossword. Tom is also thanked for his continual advice and assistance. John Lewis (Defence Science and Technology Organisation) is gratefully acknowledged for algal identification and advice on algal taxonomy. The staff of Salmon Enterprises of Tasmania (Saltas) (Sven, Peter, Harry, Pip, Jeremy, Dave, Eric, and Sid) are thanked for their assistance, enthusiasm and advice. Pheroze Jungalwalla (Saltas) is thanked for initiating, and making possible, the research projects in this thesis.

The team of the Cooperative Research Centre (CRC) for Aquaculture's Biofouling Project (Peter Rothlisberg, Peter Steinberg, Rocky de Nys, Gregor Christie and Victor Christov) are acknowledged for their expertise and enthusiasm. In particular, Rocky de Nys and Peter Rothlisberg are thanked for their support and encouragement. Dr. Greg Maguire (University of Tasmania), Dr. David Ratkowsky (University of Tasmania) and Dr. Alan Butler (CSIRO) are thanked for advice on experimental design and biostatistics.

David Foster, Philip Power and Philip Ross are thanked for assistance with field trips, and Dave in particular for adding a sense on unexpectedness to diving. Mark Hunt is thanked for listening to my many theories on biofouling, and for providing an adequate level of competition during much needed study breaks. I am grateful to Andrew Bissett for enduring long hours of sorting and weighing of fouling samples. Tiina Hawkesford generously provided a continuous supply of confectionary during the writing of this thesis. The staff and postgraduate students of the Department of Aquaculture, University of Tasmania, are thanked for their support and assistance during my studies.

International Paints are thanked for provision of silicone coatings, and John Millet (International Paints) for assistance in the coating of netting. I am grateful to the Huon Aquaculture Company for providing a field site and assistance during the last 12 months of antifouling trials. Graeme Johnson Netmakers generously supplied the netting material needed for all trials.

The development and evaluation of *in situ* cleaning equipment was funded by Saltas. The evaluation of silicone-coated netting, and the development and evaluation of antifouling polymers was funded by the CRC for aquaculture. My first year of study was supported by a University of Tasmania Postgraduate Research Award funded by Saltas, and the final 2 years by an Australian Postgraduate Award which was supplemented by the CRC for Aquaculture.

I am grateful to my parents, particularly my mother Elizabeth for her continued and unselfish support throughout my many years of study. Finally, my sincere thanks go to Nicola, Joshua, Chloe and Monique for their love and patience.

Table of Contents

CHAPTER 1

Biofouling and antifouling of finfish cages

1.1 Introduction to this study

Biofouling presents a severe operational problem to mariculture ventures. It jeopardises the health of cultured species and increases the risk of equipment damage. On fish cages biofouling restricts the flow of water through netting which reduces the supply of dissolved oxygen and the removal of excess feed and waste products (Cheah & Chua, 1979; Lee *et al.*, 1985). In suspended shell-fish culture a large mass of fouling can compete with the cultured species for food and space, and can overwhelm the flotation capacity of suspension systems (Arakawa, 1973). Fouling can also present a disease risk because the extensive communities of micro and macroorganisms may act as reservoirs for pathogens (Paclibare *et al.*, 1994). Structural fatigue and damage during storms is also exacerbated by fouling, because of its large weight (Huguenin & Ansuini, 1978) and the increased drag force on netting (Milne, 1970).

In the Tasmanian (Australia) Atlantic salmon industry the problem of biofouling is particularly severe, as nets must be removed for cleaning every 5 to 8 days during summer (Hodson & Burke, 1994). Net changing incurs a major cost to the industry, necessitating purchase of a large number of nets and provision of dedicated net-changing and cleaning teams. Handling and cleaning procedures are labour and capital-intensive and cause damage to nets. Frequent net changing risks damage and loss of stock, and disturbs feeding regimes causing lower growth rates.

Fouling protection for fish cages has been partly achieved through use of toxic, copper-based antifoulants (Lewis, 1994b). However, the use of these products is undesirable because of possible environmental effects from metal-based toxins, and because of consumer concerns that may jeopardise market image. To develop alternative and environmentally-acceptable fouling control methods the Tasmanian salmon industry initiated two antifouling research programs between 1992 and 1994. These were: (1) a short-term project for the development of an automated underwater net cleaner, and (2) a long-term project for the development of non-toxic fouling-deterrent coatings and materials. This thesis comprises the design, construction and analysis of field trials for

these projects. All trials were located within commercial fish farms to trial strategies under "in-use" conditions.

Development of a device for *in situ* mechanical cleaning of nets was considered, by industry, to be the fouling control method with the greatest likelihood of providing a short-term solution to prolong the immersion period of nets (Doedens, 1992). The project proceeded in two stages: (1) a diver-operated, hydraulic brush-test unit was constructed to allow preliminary studies of the efficacy of cleaning nets with rotating brushes, and (2) a large-scale prototype cleaner was designed (based on parameters identified from operation of the brush-test unit) and constructed. The prototype was designed to automatically track around the circumference of a cage and to clean netting to a depth of 4 m.

In 1994 a collaborative project was commenced under the auspices of Australia's Co-operative Research Centre for Aquaculture to develop antifouling coatings and materials for fish cages. The project involves collaboration between 4 groups: the University of Tasmania, University of New South Wales, the Commonwealth Scientific and Industrial Research Organisation's (CSIRO) Division of Fisheries and the CSIRO's Division of Materials Science and Technology. To date, this project has focussed on incorporating commercial short-lived biocides and compounds isolated from marine algae into solid polymers (plastics) that can be extruded as filaments for fish-cage netting. To develop material with the minimum effective release rate for each compound, and therefore ensure the longest antifouling life-time, 8 types of polymer have been trialed. Release rates have also been controlled by modifying the initial concentration of compound incorporated into the polymers.

1.2 Structure of this thesis

The first chapter of this thesis provides a review of the current literature on fouling of netting and finfish cages. Chapters 3, 4 and 5 describe the evaluation of three alternatives to traditional toxic antifouling: (1) underwater cleaning, (2) a "foul-release" silicone coating, and (3) extruded polymers incorporating naturally-occurring compounds or commercial biocides. Prior to evaluation of underwater cleaning techniques it was necessary to develop a technique to quantify the occlusion of netting mesh and to gain an understanding of the variability with the fouling communities. The development of this technique is described in Chapter 2. Chapter 6 summarises the findings of this research and discusses the potential for fouling control on finfish cages. Recommendations for effective biofouling control and directions for future research are also identified.

The following review is divided into three main sections, *viz.* (1) the effect of fouling on finfish culture, (2) the ecology and biology of fouling on netting and cages, and (3) methods for the removal and prevention of fouling on finfish cages. In comparison with research into fouling of ship hulls, oil platforms and many other marine industries, studies of fouling in finfish mariculture are limited. Moreover, many large studies specifically aimed at fouling of fish cages have been conducted by postgraduate students and are not readily available (eg. Wee, 1979; Mak, 1982; Gormican, 1989; Cronin, 1995). Many practical projects aimed at increasing mariculture productivity are conducted directly by farms, or small groups sponsored by farms, and are rarely published. Notwithstanding these limits on publishing, there is currently a good state of knowledge on the effects of mariculture fouling and its occurrence. However, techniques for control of fouling in mariculture are in their infancy, and considerable research is needed.

1.3 The effects of fouling on finfish cages

Cages for intensive commercial finfish culture are typically multifilament netting-bags suspended from a floating frame. Circular cages of 40 to 70 m circumference are the most common design, but larger 80 m cages are used for salmon culture in Australia, 90 m to 120 m cages for salmon culture in Norway and 125 m to 160 m cages for tuna culture in Australia. Square cages are also frequently used, and are produced commercially in a range of sizes from 6 m^2 to 25 m^2. The depth of cages is limited by the cage diameter, depth of the farm site and ease of maintenance, but typically varies from 10 to 20 m. The stocking density of cages is dependent on the cultured species, cage size

and environmental conditions. In Australia for example, Atlantic salmon are cultured at 10-15 kg/m^3 (eg. 12,000 X 2.5 kg salmon in a 65 m cage) and bluefin tuna at 4 kg/m^3 (eg. 2000 X 23 kg tuna in a 125-160 m cage). Given these practices, it is evident that farms using a high number of cages are required to manage a significant volume of enclosed water and large populations of fish. Good husbandry techniques are required to maintain optimum culture conditions, and protect such a sizeable monetary investment.

1.3.1 Restriction of water exchange

The predominant concern with fouling of fish cages is the occlusion of netting mesh and the resulting changes in water quality from restriction of water flow. A number of authors have measured changes in current flow through cages, and have demonstrated the extent of flow restriction through clean and fouled mesh (*eg.* Hisaoka *et al.*, 1966; Wee, 1979). The flow of water through cages is generally measured as transmission: the current speed inside the cage expressed as a percentage of the current outside the cage. The transmission of clean nets is related to mesh size, but typically varies from 50% to 80% (Table 1). The transmission is also affected by the external current velocity (Edwards & Edelsten, 1976) and the angle of the mesh to the current flow (Gularte & Huguenin, 1984). Differences in measurement of transmission may arise from the method used to quantify current, the stocking density of the cage and circulating currents created by the fish (Inoue, 1972; Wee, 1979).

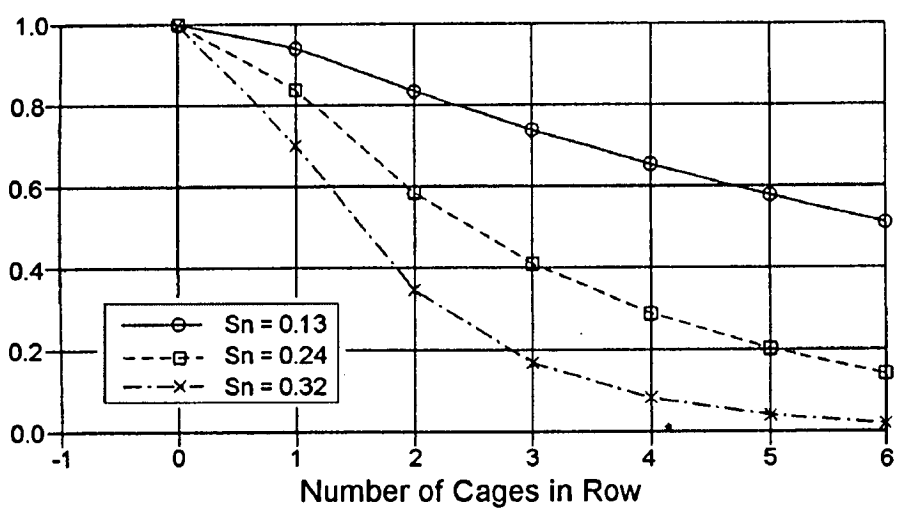
Table 1. Transmission of water through clean fish cages

Transmission (%)	Mesh size size (mm)	External current (cm/s)	Stocking density*	Cage size (m)	Author(s)
60.3	5	0.5 - 7.7	6300 (12g)	5 X 5 X 4.5	Hisaoka *et al.*,
70.2	30	1.6 - 14.3	1300 (209g)	5 X 5 X 4.5	1966
69	9	1.1 - 1.4	0	3 X 3 X 3	Inoue, 1972
80	24	1.1 - 1.4	0	3 X 3 X 3	
50	24	4 - 10	5000 (290 g)	6 X 6 X 6	
66	24	0.7 - 1.9	270 (310 g)	3 X 3 X 3	
50	16	low	0	4 X 4 X 4	Edwards &
75	16	high	0	4 X 4 X 4	Edelsten,
1976					
57.5	13	0.33 - 29.41	0	4 X 4 X 3.4	Wee, 1979
47.2	13	0.33 - 4.62	0	4 X 4 X 4	
54.2	25	0.7 - 4.78	0	8.8 X 44 X 4.9	

* number of fish (average mass)

Transmission has been shown to significantly reduce with fouling of mesh and grouping of cages. Wee (1979) found transmission for clean 13 mm mesh (57.5%) to be reduced to 23.4%, 18.7% and 13.1% after 52, 80 and 120 days in the sea, corresponding to fouling weights of 1.85 kg/m^2, 2.84 kg/m^2 and 4.98 kg/m^2 respectively. Similarly, Gormican (1989) measured current speed inside and outside a salmon cage and found a 65% transmission decrease at depths of significant fouling. Inoue (1972) showed that flow decreases serially when cages are grouped in a row parallel to the current. Across three 9 mm mesh cages the transmission was found to drop from 70% in the first cage to 35% and 18% in the second and third cages respectively. Across three 24 mm mesh cages the transmission was found to drop from 80% in the first cage to 50% and 35% in the second and third cages respectively. When cages are aligned in a series, and when netting becomes fouled, the effects combine to reduce water exchange (Fig. 1) (Aarsnes *et al.*, 1990). Beveridge (1996) thus recommended that although groups of 8-10 cages may be oriented perpendicular to the current, there should be no more than 2 or 3 cages in a series parallel to the current. The significant flow restriction through clean nets and grouped cages, necessitates good fouling control in order to maintain adequate water exchange.

Figure 1. Reduction in current velocity along a row of cages with varying levels of fouling. Reproduced from Aarsnes *et al.* (1990).



Sn: solidity factor = $2.0\frac{t}{D_t}$ where t is twine diameter and D_t is mesh size

1.3.2 Effect of water exchange on water quality

Water exchange is critical for replenishment of dissolved oxygen and removal of excess feed and waste products. A reduction in oxygen concentration from the outside to the inside of cages, and a relationship between oxygen reduction and short-term water exchange, has been demonstrated in many studies (*eg*. Hisaoka *et al.*, 1966; Inoue, 1972; Wee, 1979). Also, increasing stocking density increases the rate of oxygen consumption in cages (Kadowaki *et al.*, 1978). Consequently, under situations of low current flow and significant mesh occlusion, a high stocking density of fish may rapidly reduce dissolved oxygen to critical levels (Edwards & Edelsten, 1976). Kennedy *et al.* (1977) reported fish mortality due to anoxia in a heavily fouled cage in which the dissolved oxygen (DO) concentration fell below 4.0 mg/l. This low DO concentration was directly attributed to poor water exchange, and was increased to 8.25 mg/l after installation of a clean net. Oxygen concentrations of > 7 mg/l are recommended for salmon farming, whilst concentrations < 5 mg/l negatively impact on fish growth and respiration, and levels < 2 mg/l can result in mortality (Boyd, 1982).

A number of factors contribute to total supply and consumption of dissolved oxygen within sea cages, and the relative importance of these has been calculated through modelling (Edwards and Edelsten, 1976; Silvert, 1992; Løland, 1993a, b; Silvert, 1994; Cronin, 1995). Oxygen is primarily consumed by the fish, but to some extent also by the biochemical oxygen demand of the immediate environment and the fouling communities. Oxygen supply is largely through water exchange, but also from photosynthetic fouling communities and atmospheric diffusion. The most important factors are the respiratory demands of the fish and the mass water exchange, so that the maximum stocking density of fish is almost completely dependent on water exchange. The models allow calculation of maximum stocking densities based on the rates of oxygen consumption and supply, and also allow calculation of tolerable mesh occlusion levels for existing stocking densities. For example, Cronin (1995) found that commercial tuna cages (30 m diameter, 15 m deep, 800 mm mesh, 840 X 25kg tuna) require a transmission of at least 42% in spring (15°C water) and 80% in summer (22°C water) to maintain satisfactory oxygen levels. These latter figures also demonstrate the effect of decreased oxygen solubility with increased water temperature. However, it is likely that minimum transmission rates were

underestimated in Cronin's (1995) model because respiration rates were based on salmonids which are significantly lower than that of tuna.

Whilst oxygen levels within cages are primarily controlled by water exchange, oxygen production or consumption by fouling communities can affect oxygen concentration (Wildish *et al.*, 1993; Cronin, 1995). Wildish *et al.* (1993) recorded diurnal changes in oxygen concentrations at salmon farms, and suggested that respiratory activity of phytoplankton and fouling macroalgae significantly affected cage oxygen concentration. Gormican (1989) also observed diurnal variations in DO concentration of surface waters at salmon farms, and attributed the trend to phytoplankton. Cronin (1995) found fouling communities on tuna cages to be net consumers of oxygen, because of a greater proportion of non-photosynthetic to photosynthetic biomass. However, Cronin (1995) stated that the fouling community had minimal impact on the cage oxygen levels (less than 2% of the total oxygen exchange) relative to the processes of fish and sediment respiration and of mass water exchange.

A reduction in water exchange may also impact on fish health because ammonia can accumulate within cages. Detrimental levels of ammonia have not yet been reported in sea-cages because of sufficient water exchange (Gormican, 1989; Wildish *et al.*, 1993), but this is potentially a problem and acute ammonia toxicity has caused mortality in salmonids farmed in ponds (Lumsden *et al.*, 1993). Gowen and Bradbury (1987) estimated that 78% of nitrogen consumed by salmon is lost as faecal and excretory nitrogen. This equated to 32 kg of ammonium produced per tonne of fish food consumed. Wildish *et al.* (1993) estimated that a 450 m^3 cage, holding 8 t of fish, would produce 1120 mg ammonia/m^3/h over an average 8 month growing season. Not surprisingly, increased levels of ammonia have been found within cages, compared to surrounding waters (Gormican, 1989; Wildish *et al.*, 1993). The toxic effects of unionised ammonia (NH_3) vary greatly with species and environmental conditions. The recommended maximum concentration for salmonids is 0.02 - 0.025 mg/l (Solbé, 1988; Branson, 1993), and long-term exposure to concentrations greater than this can cause sub-lethal effects such as reduced growth or immuno-competency (Alabaster & Lloyd, 1982). The combination of ammonia accumulation and oxygen depletion may enhance detrimental effects. Decreased oxygen levels may reduce ammonia tolerance (Knoph & Thorud, 1996), and high levels of ammonia can interfere with oxygen uptake (Branson, 1993).

1.3.3 Cage deformation and structural fatigue

An increase in mesh occlusion will significantly increase drag forces on netting. Milne (1970) determined current forces on clean and fouled nets at various current velocities, and showed that forces on a fouled net may be 12.5 times that of clean net. Consequently, unless cages are heavily weighted the shape of the cage may be severely deformed by current flow (Osawa *et al.*, 1985). Aarsnes *et al.* (1990) calculated deformation rates for a 12,000 m^3 cage (with 400 kg of bottom weight) and found that the cage volume was reduced by 45% (to 6,600 m^3) under a current velocity of 0.5 m/s (1 kn), and by 80% (to 2,300 m^3) under a velocity of 1 m/s (2 kn). Wee (1979) observed a 50% reduction in volume of a heavily fouled in use cage. Reduced cage volume is likely to impact on fish health because oxygen consumption and ammonia production will increase per unit volume, and crowding is likely to stress the cultured fish.

Highly deformed nets increase the structural stress of the cage and, although increasing cage weight will reduce deformation, this adds to the structural stress (Anon, 1993). Tomi *et al.* (1979) reported that weight added to cage corners resulted in a two- to six-fold increase in horizontal forces on the cage. With heavy weighting, waves will cause the floating frame to move upward whilst the weights pull the netting downward. Structural loadings and fatigue are likely to increase further when predator netting is attached to cages.

1.3.4 Disease risk

Fouling communities may present a health risk to the cultured species because they can act as reservoirs for pathogenic microorganisms. These pathogens may be carried and released by macrofouling species, or exist in the extensive microbial communities reported on cage netting (eg. Hodson & Burke, 1994). Viral pathogens of finfish were reported by Meyers (1984) to accumulate and persist for long periods within shellfish. Meyers (1984) further reported that a number of bacterial agents that cause disease in finfish are also common to bivalves tissues (eg. *Vibrio* sp.). Paclibare *et al.* (1994) evaluated the potential of the bacterial kidney disease agent *Renibacterium salmoninarum* to be concentrated in the mussel, *Mytilus edulis*. *R. salmoninarum* is shed in the faeces of infected salmon, and it was considered that filter-feeding bivalves (which foul the net cages) may accumulate the pathogen and act as a continuous source of re-infection. However, the majority of cells were killed during digestion so that mussels were unlikely

to serve as a long-term reservoir. Mussel fouling could, therefore, be beneficial for the reduction of pathogenic bacteria.

The occurrence of disease in caged fish has also been linked to the consumption of fouling organisms by the cultured species. Kent (1992) suggests this method of transfer for both 'netpen liver disease' and infection by *Gilquinia squali* metacestodes. Netpen liver disease (NLD) is thought to be caused by a hepatotoxin which may be produced from an alga abundant in sea cages during summer. Fish observed to feed heavily on herbivorous amphipods from the cages often develop NLD. Consumption of fouling crustaceans is likely to be the cause of infection by the trematode *G. squali*. It is likely that, during one of its life-stages, a crustacean acts as an intermediate host, and that transfer to the definitive host (a fish) occurs directly through ingestion (Kent, 1992).

In summary, the occlusion of netting mesh and the resulting restriction in water exchange, can adversely affect fish health by the reduction in DO and potentially by accumulation of metabolic ammonia. Fouling is of further concern because it significantly decreases cage flotation, increases structural fatigue and cage deformation, and may act as a reservoir for pathogens. Consequently, research to identify the composition and development of cage fouling, and methods for the removal and prevention of fouling, is essential to the industry.

1.4 Biofouling development and ecology

Fouling of mariculture structures differs from that of many other marine industries in terms of surface characteristics, environmental conditions, adverse effects which result, and the methods suitable for fouling control. Surfaces most affected are multi-filament netting, extruded plastic mesh and shells. These structures are typically rough, non-toxic, and rarely coated with antifoulants. They are also not subject to the high water velocities associated with ship hulls or the internal surfaces of pipes. Fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by the high-density fish populations (Gowen & Bradbury, 1987). Furthermore, spores and larval stages of fouling organisms are likely to be abundant at farms because cage systems constitute a massive surface area on which fouling communities reside.

The first studies of fouling on mariculture netting were published in the 1960's and 70's, and were aimed at identifying netting material suitable for fish enclosures (eg. Milne & Powell, 1967; Rothwell & Nash, 1977). This research showed netting material and mesh size to significantly affect fouling rate, mesh occlusion, and density and abundance of fouling species. Furthermore, spatial and temporal variation in net fouling was demonstrated over a wide geographical range, with test sites located in Scotland (Milne & Powell, 1967; Milne, 1969, 1970, 1975a, b), Hawaii (Rothwell & Nash, 1977), Hong Kong (Mak, 1982), Maine and Massachusetts (Huguenin & Ansuini, 1975, 1977, 1980, 1981, 1984). From this data, and observations of mesh deterioration, materials were rated for their suitability in the construction and maintenance of fish cages. More recently, the effects of net angle (Cheah & Chua, 1983) and of microfouling development on multi-filament mesh (Hodson & Burke, 1994) have also been investigated.

1.4.1 Effect of mesh size

A variety of mesh sizes are employed for commercial finfish culture, ranging from 12-40 mm for salmon cages, 60-90 mm for Bluefin Tuna cages to 100-150 mm for predator fences. The larger meshes are often of thicker gauge, but generally the smaller the mesh size the greater the surface area per m^2. Consequently, smaller meshes typically support a greater number of fouling organisms and total biomass (Milne, 1975a; Cheah & Chua, 1983). Cheah and Chua (1983) found the rate of fouling, mass of fouling, species diversity and species abundance to increase with a decrease in mesh size. For example, mesh sizes of 38 mm, 25 mm and 13 mm were fouled by 1, 3 and 5 species of colonial ascidian respectively. Small mesh sizes are also blocked by a relatively low mass of fouling, whereas larger mesh material (>50 mm) can support large fouling communities but maintain a significant open area (Milne & Powell, 1967). Consequently, to maintain acceptable water exchange small mesh nets must be cleaned far more frequently than larger meshes (Cheah & Chua, 1983).

Small mesh netting ($\leq$ 15 mm) is particularly prone to accumulation of suspended sediment, and often has significant differences in fouling for this reason alone. Mak (1982) found 9 mm mesh supported less biomass than 63 and 88 mm mesh and attributed this to a high accumulation of sediment which completely blocked the small mesh. Similarly, Lai *et al.* (1993) found 10 mm mesh supported almost twice the fouling biomass of 1 mm mesh, but only the latter had a heavy accumulation of silt and mud.

However, Cheah and Chua (1979) found high silt loadings on nets provided an excellent substrate for settlement and growth of fouling, particularly *Gracilaria.* The accumulation of sediment is exacerbated by the rough surface of multifilament mesh. Lee *et al.* (1985) quantified sediment loading on 13 mm, 9 ply mesh, and found 3.02 kg/m^2 on an untreated net compared with only 0.05 kg/m^2 and 0.06 kg/m^2 on nets coated with an antifoulant.

Comparisons between different mesh sizes are affected by twine thickness because this changes the total surface area. Mak (1982) quantified fouling on mesh panels after 3, 6 and 9 months immersion, and found 25 mm and 50 mm multifilament meshes supported a greater biomass than 9 mm, 63 mm and 88 mm single-filament meshes. Tseng and Yuen (1979) found no significant difference in fouling mass on 50 mm, 38 mm, 20 mm and 19 mm mesh nets, which were woven from 36, 27, 9 and 4 filaments, respectively.

Whilst short-term fouling development (< 3 months) appears dependent on available surface area, the literature also suggests long-term fouling mass (particularly of filter-feeding invertebrates) is dependent on the area in which the organisms can expand and feed. Mak (1982) found smaller meshes supported the greatest fouling biomass after 3 months immersion, but larger meshes supported the greatest biomass after 9 months immersion. These communities were dominated by invertebrates and more than 75% of the 9-month community was composed of solitary ascidia. Similarly, Milne (1975a) found large mesh sizes eventually developed mussels of a greater size than small mesh, and suggested that the water flow through larger mesh improved feeding.

1.4.2 Effect of mesh structure

The microtopography of multi-filament netting affects the distribution and type of initial fouling (Hodson & Burke, 1994). The cylindrical shape of mesh bars leads to differences in light intensity between the upper and lower surfaces of bars immersed horizontally. Consequently, horizontal bars develop a community dominated by phototrophs (eg. diatoms) on the upper surfaces, and heterotrophic protozoan communities on the lower surfaces (Hodson & Burke, 1994). The large crevices and many filaments of the netting are likely to aid colonisation, either through entrapment of suspended material or because larvae of some fouling invertebrates, and spores of common fouling organisms such as *Ectocarpus* and *Enteromorpha*, preferentially settle in small depressions (Crisp, 1984). The use of monofilament netting would obviously reduce problems associated with crevices, but it has significantly less strength than multifilament

mesh. Furthermore, monofilament nets must be constructed with knots at the mesh intersections, and this results in increased abrasion damage to nets during on-shore handling and increased abrasion of fish during culture.

Fouling development on netting is influenced by the 3-dimensional structure of mesh. Preferential colonisation at mesh intersections has been noted in many studies (eg. Milne, 1975a, b; Rothwell & Nash, 1977; Tseng & Yuen, 1978). Milne (1975a, b) observed that mussels developed large aggregations at intersections, and Tseng and Yuen (1978) reported bryozoans, barnacles, and green algae primarily occurred at knotted intersections. This preferential settlement presumably results from the greater surface area and changes in turbulence at these regions. Milne (1975b) also noted that the netting structure led to entanglement of drifting algae. This type of fouling can quickly block netting, and is unaffected by antifoulants because it is entangled rather than directly attached to the surface.

1.4.3 Effect of mesh material

A number of materials are suitable for the construction of fish cages, and these have varying degrees of fouling resistance. In this regard several studies have demonstrated the relative performance of many types of netting: multifilament-polymer mesh, extruded polymer mesh, metallic hardware cloth, and extruded metallic mesh (eg. Milne & Powell, 1967, 1969; Rothwell & Nash, 1977; Huguenin & Ansuini, 1981, 1984).

Milne and Powell (1967, 1970) compared 10 mesh types at 4 sites in Scotland, and found polymer-fibre nets were the most susceptible to fouling and galvanised meshes the least. After 4 months immersion mussel growth (*Mytilus edulis*) completely blocked polymer-fibre netting and the weight of test panels (0.4 m^2) had increased from 5.5 kg (clean) to more than 15.5 kg. In comparison, reasonable water flow still occurred through galvanised materials, and panel weight had increased from approximately 7 kg to 9 kg.

Rothwell and Nash (1977) assessed 9 types of netting in a 6-month trial at 3 locations in Hawaii. Netting panels were compared by initial fouling rates to determine time interval before cleaning, and by the total fouling mass after 5 months. Nylon and polyethylene meshes were found to foul at a significantly greater rate than metallic meshes, and after 5 months polyethylene mesh had the greatest fouling and galvanised mesh the least. The composition of the fouling community also differed between mesh types. Initially algae colonised the majority of net types, but became most abundant on

nylon netting and netting with an ineffective antifouling paint. After 5 months, serpulid tubeworms were abundant on all panels, but were least prevalent on extruded polymer mesh and PVC-coated chain-link on which barnacles were abundant.

The suitability of rigid Cu/Ni mesh, and its inherent antifouling ability, was evaluated by Huguenin and Ansuini (1975, 1977, 1980, 1981, 1984). Biofouling development was monitored at 2 sites in the USA, to compare 90-10 Cu/Ni mesh, Al-bronze mesh, and galvanised and polymer mesh. Polymer meshes were heavily fouled by mussels and ascidians within 4 months, and were more than 70% occluded. Galvanised mesh provided some fouling inhibition, but became severely fouled after 8-9 months immersion. The Cu/Ni mesh had excellent fouling resistance and was never more than 10% blocked for 25 months at one site, and for 10 months at the other site. The shorter duration at the latter site resulted from a bloom of filamentous diatoms which blocked all mesh types. Fouling composition was also compared, and metallic meshes supported both a lower mass and diversity of fouling than polymeric meshes. Of the metallic meshes, Cu/Ni was considered superior because poor galvanisation caused the galvanised mesh to disintegrate within 20 months.

The fouling resistance and mechanical strength of galvanised and Cu/Ni meshes led to a number of economic analyses of rigid metallic mesh versus flexible synthetic mesh (eg. Huguenin & Ansuini, 1975). A comparison of the degradation rates, corrosion, and loss of material strength over time for various materials was discussed by Milne (1970). Whilst it was recognised that metal mesh had a significantly higher purchase price than synthetic meshes, it requires less maintenance and has a longer service life. Consequently, over a 10 year period Cu/Ni cages and barriers were estimated to be of comparable or lower cost than synthetic material (Huguenin & Ansuini, 1975). However, present day cages almost exclusively use multi-filament (typically nylon or high-density polyethylene) netting mesh. This material offers high strength, reasonable durability, ease of handling, and is economically acceptable. Furthermore, it can be easily applied to many cage designs, particularly very large cages (eg. 120 m circumference circular cages) which would be difficult to construct and move if fabricated with rigid metallic mesh.

1.4.4 Fouling composition and biomass

The development and composition of fouling communities on fish cages have been described for many types of mariculture in a number of countries, including Australia

(Cronin, 1995), China (Chengxing, 1990), India (Santhaman *et al.*, 1983), Japan (Kuwa, 1984), Malaysia (Cheah & Chua, 1979; Lee *et al.*, 1985), Tanzania (Bwathondi & Ngoile, 1982) and the USA (Moring, 1973; Moring & Moring, 1975). There are also some studies of cage fouling in freshwater ponds and lakes (eg. Pantastico & Baldia, 1981; Greenland *et al.*, 1988; Dubost *et al.*, 1996). This research has shown that significant differences in fouling occur at different depths and sides of cages, and between adjacent cages within a farm. The studies have often recommended frequency schedules for onshore net cleaning at different sites, and identified those fouling organisms of particular concern. The fouling communities have also been considered in terms of their importance as supplemental food for the cultured fish (Moring & Moring, 1975; Black *et al.*, 1992).

The majority of studies of fish-cage fouling are long-term analyses, giving data on species diversity and abundance over months and sometimes years (eg. Milne, 1975a, b; Moring & Moring, 1975; Cheah & Chua, 1979; Lee *et al.*, 1985). These immersion periods provide useful data on succession in fouling communities and long-term performance of antifoulants or netting materials. However, such time periods are not typical of many mariculture operations. In practice netting is changed and cleaned often (eg. every 1-4 weeks), either when water flow is restricted, or before the weight of fouling becomes too great for easy removal of the net for cleaning (see 1.5.1).

Fouling communities on cages are often characterised by a high species diversity and a large biomass/m^2. Biomass in the range of 1-5 kg/m^2 (wet weight) is typically reported (eg. Lee *et al.*, 1985; Cronin, 1995). This constitutes a significant weight since a 65 m circumference 10 m deep net would have a surface area of 986 m^2 (including a flat base panel). For example, Cronin (1995) estimated a mean biomass of 4-5 kg/m^2 on tuna cages, and this equated to a total mass of 6.5 t on a 90 m circumference net. Wee (1979) quantified biomass change over time, and found an increase from 1.85 kg/m^2 to 2.84 kg/m^2 and 4.98 kg/m^2 after 52, 77 and 106 days respectively. Similarly, Cheah and Chua (1979) found a 4-month community having nearly an identical species composition to a 2-month community, but double its weight. Lee *et al.* (1985) demonstrated a large increase in netting weight from silt deposition, with a total fouling mass of 4.5 kg/m^2 of which 58% was silt. Chenxing (1990) found that the biomass of the ascidian *Styela picata* reached 5.3 kg/m^2 in one season. Rothwell and Nash (1977) reported a total fouling mass

of 13 kg/m^2 on nylon netting after 1 month in Pearl Harbour, but in excess of 80 kg/m^2 after 3 months. Similarly, Milne (1975a) found that 25 mm nylon mesh could support a mussel biomass of up to 140 kg/m^2.

Macroalgae are the most serious type of fouling on cages immersed for short periods (< 1 month) in Tasmania, Australia (Hodson & Burke, 1994), whilst bivalves and ascidians are predominant on cages immersed for longer periods (Bwathondi, 1985). In some countries invertebrates can also cause significant fouling in short periods, particularly during times of high larval settlement (Sutterlin & Merrill, 1978). On cages in-use a larger biomass of invertebrates is often better tolerated than algae. A small algal biomass can significantly occlude mesh, whereas a comparable level of blockage will only occur with large communities of bivalves or solitary ascidians. However, the water mass within solitary ascidians and the shell mass of bivalves dramatically increase cage weight.

The dominant macroalgae reported on fish cages include *Gracilaria* (Cheah & Chua, 1979), *Ulva* (Moring & Moring, 1975; Cronin, 1995), *Antithamnion* (Hunter & Farr, 1970) *Enteromorpha* and *Ectocarpus* (Wee, 1979; Santhaman *et al.*, 1983). Significant mesh occlusion by filamentous (tube-dwelling) diatoms has also been reported (eg. Moring & Moring, 1975; Huguenin & Ansuini, 1984; Hodson & Burke, 1994). Bivalves reported as major net-cage foulers include the wing shell *Electroma georgiana* (Cronin, 1985), the mussels *Mytilus edulis* (Koops, 1971; Moring & Moring, 1975; Paclibare *et al.*, 1994), *Modiolus* sp. and *Perna viridis* (Cheah & Chua, 1979; Lee *et al.*, 1985), and the oysters *Crassostrea* spp and *Pinctada* spp (Cheah & Chua, 1979; Bwthondi & Ngoile, 1982). The major fouling ascidians include solitary species such as *Styela picata* (Chenxing, 1990), *Ascidiella aspersa* and *Ciona intestinalis* (Milne, 1975b), and colonial genera including *Botryllus, Botrylloides, Symplegma* and *Tridemnum* (Cheah & Chua, 1979).

The rapid growth of fouling on fish cages results from increased carbon, nitrogen and phosphorus levels in the waters immediately surrounding mariculture farms. Farm waters are enriched with organic and inorganic wastes, from uneaten food and from faecal and excretory material from the high-density fish populations (Gowen & Bradbury, 1987). These forms of nutrient enrichment have been shown to directly increase growth of fouling algae in laboratory studies (eg. Brault & Quéguiner, 1989) and field trials (eg. Rothwell & Nash, 1977). Ruokolahti (1988) found nutrient enrichment to increase

biomass, length and chlorophyll *a* content in populations of *Cladophora glomerata* (Chlorophyceae) near fish farms. Rothwell and Nash (1977) observed rapid fouling growth in the nutrient enriched waters of Pearl Harbour and the complete blockage of netting mesh within 2 months. In comparison, they found the majority of panels immersed at 2 sites with minimal nutrient enrichment had only 0-10% blockage after 3 months immersion.

1.4.5 Variability in fouling communities

Fouling mass and species diversity have been found to vary between sides of cages, primarily because of differences in exposure to direct sunlight. Moring and Moring (1975) observed significant variation between cage sides, but this decreased with depth. Significant variation occurred between sides at 0.5 m, some variation at 1.0 m, but there was no significant variation at 2.0 m. This trend was directly attributed to light intensity. Similarly, Lee *et al.* (1985) observed significant differences in the mass of algae and invertebrates between cage sides. They found the two faces that had the greatest mass of bivalves (*Modiolus* sp. and *Perna viridis*), had the lowest mass of marine worms and algae. Cronin (1995) found the southern side (which received direct sunlight) of a tuna cage had a greater photosynthetic biomass than other sides, and the highest total biomass over most depths. Significant differences between sides were also noted for specific organisms. Ascidians comprised a greater proportion of the community on the southern side than any other side, and rhodophytes were more abundant on the southern side than any other side. However, bryozoans were least abundant on the southern and western sides.

Reduction in light intensity also causes significant differences in species diversity and abundance between depths. The depth of fish cages ranges from just 2 m (Lee *et al.*, 1985) up to 20 m (Hodson & Burke, 1994). Deeper cages (10-15 m) are typical for large-scale finfish culture. Moring and Moring (1975) showed fouling mass to significantly decrease with increasing depth (from 0.5 m, 1 m and 2 m). Wee (1979) found the top metre of a salmon cage to be fouled with *Ectocarpus*, *Enteromorpha* and other algae, whilst *Mytilus edulis*, hydroids and amphipods were dominant at lower depths. Similarly, Santhanam *et al.* (1983) reported the upper third of a cage to have the greatest algal fouling, and the lower two-thirds to have the greatest oyster settlement. Cronin (1995) identified fouling mass and composition at 1, 2, 3, 5, 8 and 12 m on each

side of a tuna cage. The organism contributing the greatest biomass was the bivalve *Electroma georgiana*, and its abundance was found to increase with depth.

Increased fouling growth around the top of cages, particularly of algae, is also shown by measurements of mesh occlusion. Fukuda *et al.* (1965) reported fouling growth and mesh occlusion increased with distance above the base of a cage (Fig. 2). Haegele *et al.* (1991) reported a gradual decrease in mesh occlusion from 50% to 10%, over 0.3 to 9.1 m depth. Consequently, restriction in water exchange and the associated degradation in water quality are also likely to vary with depth, and could result in aggregation of the fish at specific depths to avoid unfavourable conditions (Gormican, 1989).

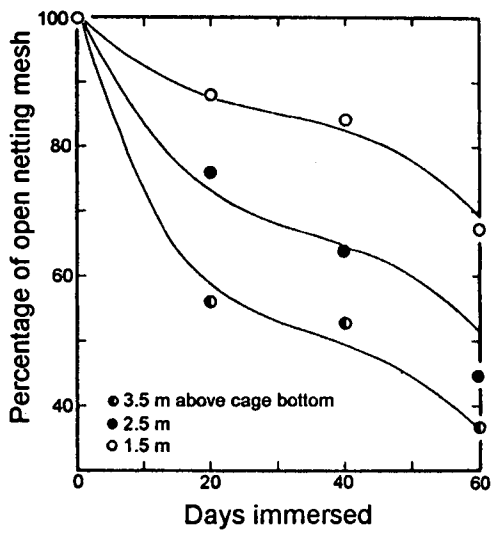


Figure 2. Reduction in mesh occlusion with depth. Reproduced from Fukuda *et al.* (1965)

The orientation of submerged surfaces affects fouling development, and significant differences occur between vertical and horizontal substrates (Harris & Iron, 1982). For example, Lee *et al.* (1985) found a greater mass of bivalves on the bases, rather than the walls, of 2 m deep cages. Cheah and Chua (1983) reported that horizontal regions of net cages had less fouling than vertical. To some extent these observations reflect a change in fouling with depth, but they also represent an orientation effect. This was demonstrated by Cheah and Chua (1983) in a comparison of vertically and horizontally mounted net panels. The vertical panels were fouled more rapidly, developed a greater mass of fouling, and had increased abundance of compound ascidians and tubeworms. However, barnacles and oysters were more abundant on the horizontal frame. The increased mass on the vertical panel was thought to reflect a greater

interception of horizontally moving planktonic larvae thus increased larval settlement. However, it is also likely that an increase in collisions with suspended material would increase nutrition of filter-feeding organisms. The fouling difference also conforms to Harris and Iron's (1982) study of the effect of substrate angle on species composition and relative abundance. Communities on horizontal surfaces are subject to greater siltation and predation than vertical surfaces, and upright or mounding species are favoured. However, colonial growth is more effective on vertical surfaces where competition for space is critical and predation pressure is less.

Variation in fouling composition has been observed between adjacent cages, and may reflect changes in current flow, exposure to direct sunlight or a difference in time of immersion. Bwathondi and Ngoile (1982) observed differences in fouling between two adjacent 0.5 m^3 cages, and between the outer and inner surfaces of these cages. After 103 days immersion 9 species of bivalve were identified, with 672 bivalves on one cage and only 315 on the other. More individuals of each species were found growing on the outer than inner surfaces of both cages. On the more heavily fouled cage, a total count of 503 and 169 bivalves were attached to the outer and inner surfaces respectively. Greenland *et al.* (1988) quantified bryozoan fouling (*Plumatella casmiana*) on 1m^3 catfish cages that were contained in a 1.1-hectare pond. Cages at the inlet end of the pond had the greatest fouling, and the extent of fouling was shown to be inversely related to pond water depth and the distance of the cage from the pond water supply inlet.

Spatial variation in cage-fouling has also been shown on a larger scale, and may represent differences in environmental conditions (Santhanam *et al.*, 1983) or abundance of larval stages (Bwathondi & Ngoile, 1982; Bwathondi, 1985). Santhanam *et al.* (1983) reported that fouling communities on polyethylene netting differed between cages immersed in brackish and marine waters. Cages in brackish water (24.5 - 33.8 ‰) were colonised by the algae *Enteromorpha* and *Ectocarpus*. However, cages in marine conditions were colonised by bivalves (*Avicula vexillum, Dasychone, Crassostrea madrasensis,* and *Pinctada*), sea anemone, solitary and colonial ascidians, algae (*Caulerpa* spp, *Codium* sp. and *Gracillaria*), amphipods (*Corophium*), barnacles (*Balanus amphitrite variegatus*), and polychaetes (*Serpula* sp.). Bwathondi and Ngoile (1982) and Bwathondi (1985) found different age classes of bivalves fouling fish cages, and identified the frequency and time of settlement of different species. They identified 8

age groups of an *Ostrea* sp., 4 groups of a *Pictada* sp. and 3 groups of *Pinctada vulgaris* on cages immersed for 103 days. The number of individual per age group was dependent on environmental conditions, and greater settlement of *Ostrea* occurred during spring tides (the time of greatest plankton abundance), and greater settlement of *Pinctada* occurred at high rainfall. Haegele *et al.* (1991) recorded the abundance of fouling invertebrates at numerous sites, and at various depths within sites, at salmon farms in British Columbia. Mussels, isopods and pycnogonids were frequently observed, but their abundance varied greatly between sites on different sampling dates. Further, species such as polychaetes which occurred in low abundance were only found at a few sites.

1.5 Methods for the control of biofouling

The significant problems associated with fouling of ship hulls, oil platforms and seawater-intake pipes, have led to research on numerous techniques for fouling removal and prevention. Experiments with antifouling of ship hulls have been attempted for centuries, with lead sheathing of hulls used as early as the 3rd-century BC and arsenic-based coatings in 412 BC (Woods Hole, 1952). During the past 50 years antifouling paints have been intensively studied, although products designed specifically for fish cages are scarce. This partly reflects the relative infancy of commercial finfish culture, but is also indicative of problems with poor paint adhesion to netting and antifoulant toxicity to cultured species. However, the continual expansion of finfish mariculture is increasing demand for fish-cage antifouling, and has expanded research opportunities and created a viable market for paint manufacturers to develop specific products.

1.5.1 Shore-based net cleaning

The removal of fouling communities from cages is generally achieved by replacing the fouled net, and transporting it to shore for manual or semi-automated cleaning (Lewis, 1994b). However, the frequent changing of netting on a standard floating cage is labour and capital-intensive, and boat-mounted hydraulic cranes are needed for large cages. During changing, the fouled net is partially raised and a clean net is peeled underneath and attached to the collar. The fouled net is then untied and removed, with the fish released into the clean cage. Fouled netting is usually left to compost for 1-2 weeks on-shore, followed by cleaning with high-pressure water hoses or automated washing machines (Lewis, 1994b; Sutterlin & Merrill, 1978). Unfortunately, washing procedures and net handling frequently cause damage to netting and reduce its life-span.

Consequently, after cleaning nets are laid out for mending and replacement of damaged sections. At some farms nets are dropped to the seabed after removal from the cage, and the fouling is degraded biologically over a period of weeks (Sutterlin & Merrill, 1978). However, this latter technique is unsuitable when clean nets must be available within short-time periods, and the practice is also likely to increase benthic pollution.

Fouling develops very rapidly on cages in many regions of the world, and frequent changing and cleaning of nets is critical to maintain cage water exchange. For example, nets must be changed every 5-8 days in summer in Australia (Hodson & Burke, 1994), every 8-14 days in Japan (Milne, 1979) every 14 days in Malaysia (Lee *et. al.*, 1985), and every 3-4 weeks in Canada (Menton & Allen, 1991). Consequently, net changing incurs a major cost to the industry, necessitating purchase of a large number of nets and provision of dedicated net-changing and cleaning teams. Moreover, frequent net changing also risks damage or loss of stock, and disturbs feeding regimes which lowers growth rates. Some delay in the frequency of cleaning may be achieved by raising the top few metres of the cage out of the water (Needham, 1988), but this is only applicable where the fouling is restricted to the upper area of the cage.

Whilst frequent net changing is common in temperate and tropical regions, cages immersed at off-shore sites and in very cold water can remain immersed for long periods without cleaning. For example, cages in northern Norway are changed only once per year, usually in July after the period of maximum ascidian and mussel settlement (Sutterlin & Merrill, 1978). Large mesh cages are also changed less frequently because of the considerable amount of fouling required to occlude the mesh significantly. In Australia predator fences (100-150 mm mesh) are changed every 3-6 months, and tuna cages (60-90 mm mesh) are cleaned every 6 months (Cronin, 1995).

1.5.2 Underwater net cleaning

Given the large expense involved in frequent net changing, it is surprising that little information is available on underwater cleaning of cages. However, many research initiatives to improve farm management are conducted by farms themselves or by engineering firms, and details are published only in industry-generated or directed literature. A recently published article in *Fish Farming International* (Anon, 1994) discussed a cleaner designed by Japan's Bridgestone Corporation and this is, perhaps, the only significant development of automated underwater cleaning for fish cages. The

machine consists of a cleaning head (with rotating brushes) supported beneath a floating platform which moves around the cage perimeter. Two cables are used for raising and lowering the cleaning head. The machine was reported to clean 4 m^2 - 6.3 m^2 of net per minute (3 hours for a 15 m deep hexagonal cage with 16 m long sides) depending on the level of fouling. However, the design does not allow for removal of debris created during cleaning. Doedens (1992) reported on an earlier version of the machine, and quoted a purchase price of Aus$166,000. At that time only 10 units had been sold in its 2 years of commercialisation.

Simpler forms of underwater cleaning are practiced, but often require SCUBA diving and are therefore more expensive and dangerous than shore-based cleaning. High pressure water hoses have been used to clean tuna cages in South Australia (Cronin, 1995), and vacuum cleaning equipment has been used for salmon cages in Tasmania (Doedens, 1992). However, the latter technique was only effective on painted nets (because the fouling attached poorly), and was eventually abandoned because of the considerable amount of time required to clean an entire cage. Hand-held units combining a rotating brush and high-pressure water jets have been offered commercially, but are probably only cost-effective for small cages. In general, *in situ* cleaning is unlikely to be viable unless fully automated; any fouling remnants left after cleaning are likely to regrow quickly and underwater cleaning may therefore be required at a high frequency (Moss & Marsland, 1976a). Geffen (1979) suggested that brushing increases fouling problems because it scratches the mesh and encourages rapid recolonisation.

1.5.3 Fouling removal by herbivorous and omnivorous fish

Fouling removal has been conducted biologically, by including low numbers of herbivorous or omnivorous fish within culture cages. Beveridge (1996) suggested that an increase in profitability and reduction in farm waste could be achieved by use of herbivorous fish to control fouling, and benthic/detritus feeders to remove uneaten food. This has previously been successful with inclusion of siganids in cages of grouper and carangids (Chua & Teng, 1977), rohu (*Labeo rohita*) in cages of carp (Sharma, 1979), and knifejaws in cages of yellow tail (Kuwa, 1984). The stocking density of the added herbivorous fish varies greatly, from 3% - 9% of the total cage biomass (Kuwa, 1984; Li, 1994) to densities of only 1 fish/5 m^3. The use of grazing finfish can also prove effective for controlling fouling of suspended shellfish systems (Hasse, 1974; Littlewood, 1990).

Furthermore, a variety of biological control methods could be used in shellfish culture, from species of snails and crabs that preferentially feed on colonial ascidians to species of nudibranch which selectively feed on *Tabularia* (Arakawa, 1973).

The addition of herbivores may present a number of risks to the primary culture species, such as greater disease potential and increased demands on dissolved oxygen. Kuwa (1984) reported that knifejaws preyed on the tail and fins of sick yellow tail. The great variation in types of algal and invertebrate fouling also suggests that only herbivores and omnivores with a broad dietary range will be successful. It is likely that continuous grazing will provide an environment selective for inedible species, and thus polyculture may only reduce the frequency of net changing.

1.5.4 Alternative cage designs

An alternative to both frequent net changing and underwater cleaning, is through use of fully-enclosed rotating cages (e.g. Caillouet, 1972; Anon, 1979; Blair & Burgess, 1979; Geffen, 1979; Blair *et al.*, 1982; Menton & Allen, 1991; Willinsky *et al.*, 1991). These have either been horizontally-mounted cylinders which rotate on a central axle (Caillouet, 1972; Menton & Allen, 1991), or rectangular boxes with inflatable buoyancy devices in each corner. The rectangular cages are gradually rotated by sequentially changing the buoyancy of the corners (either by inflation and deflation, or displacement and filling with water). With rotatable cages no area of netting needs to be left submerged for long periods, and netting can be brought to the surface to air dry and hence kill attached fouling. Furthermore, the cage is easily accessible for fouling removal and netting repair, and by keeping the net immersed for short periods significant fouling growth can be avoided. Blair *et al.* (1982) found that a cage rotation of 90 degrees per week was sufficient to keep cages essentially free of fouling, and Geffen (1979) reported that cage rotation at 3-day intervals kept cages completely clean.

Rotating cages are not widely used, even though completely enclosed cages offer other benefits such as prevention of bird predation, and avoidance of storms and ice through cage submergence. Presumably, it would be necessary to construct very large rotating cages if they were to hold volumes of fish comparable to conventional floating collars of > 90 m circumference. Moreover, commercially available rotating cages are more expensive than conventional designs and continued exposure to direct sunlight can increase netting degradation (Beveridge, 1996).

1.5.5 Fouling prevention

The application of antifoulants to fish-cage netting is practiced in many countries, and 'discoveries' of new and improved antifoulants appear almost monthly in industry publications (eg. Anon, 1996a, 1996b, 1996c, 1996d). Antifoulants designed specifically for cage netting are presently based on copper oxide and add approximately 20-25% to the cost a knotless nylon cage (Beveridge, 1996). In temperate regions nets must be coated each year, but antifouling gives good protection for 6 months and is beneficial during summer when fouling is worst (Beveridge, 1996). However, the use of toxic metal-based antifouling is frequently seen as undesirable in mariculture, particularly as the industry is selling a food product (Hodson & Burke, 1994).

Some of the earliest published attempts at antifouling of fish cages were conducted in the Western Baltic Sea by the Institute for Coastal and Freshwater Fisheries (Klust & Kuhl, 1960; Koops, 1971). Koops (1971) showed an antifoulant (Wiedox VF 65001/green) kept nets completely clean for 5 months, during which time untreated nets became totally occluded. Lee *et al.* (1985) showed significant reduction in net fouling when cages (2 X 2 X 2m) were coated with an organotin antifoulant. After 2-months fouling masses of 1 kg/net and 91 kg/net were found on treated and untreated cages, respectively. More recently, Hodson and Burke (1994) found that nets coated with a toxic copper-based paint and a non-toxic silicone coating effectively reduced the initial stages of fouling development. The silicone coating was aimed at reducing or preventing the adhesion of fouling, and is presently seen as an alternative to toxic paints for ship hulls.

The use of tributyltin (TBT) antifoulants has demonstrated the potential hazards of toxic coatings in mariculture. High levels of TBT have been recorded in the waters around antifouled fish cages (Balls, 1987). Balls (1987) measured TBT release in newly painted salmon cages, and recorded 1 mg/m^3 (µg/l as Sn), 0.1 mg/m^3, and 0.005 mg/m^3 after 1 day, 2 weeks and 5 months, respectively. Short and Thrower (1986) reported TBT concentrations from 0.007 to 0.026 mg/m^3 Sn in treated salmon pens in the USA. These concentrations have been shown to induce histopathological effects and mortality in salmonids. Short and Thrower (1986) reported a 96-h LC_{50} of 1.5 µg/l for Chinook salmon, and demonstrated rapid bioaccumulation after 3-4 days exposure to 1.5 µg/l. They recorded levels of 6.4, 1.9 and 0.3 µg TBT/g wet weight of liver, brain and muscle

respectively. Similarly, Davies and McKie (1987) showed bioaccumulation in tissues of Atlantic salmon exposed to 1 µg/l TBT for 28 days. Bruno and Ellis (1988) reported behavioural abnormalities and pathological changes in farmed Atlantic salmon that were transferred to a newly antifouled cage. Salmon showed lifting of the gill epithelium and an increase in number of leucocytes in the retina after 4 days. After 7 weeks exposure, hyperplasia was observed in the dermal layers of the skin, and TBT had bioaccumulated in the flesh, liver, gills and caeca.

The adverse effects resulting from wide-spread use of TBT have lead to a ban on its use in many circumstances. For example, in 1986 the National Farmers' Union in Scotland introduced a voluntary ban on its use in fish cages, and in 1987 its retail sale was prohibited by the Scottish government (Balls, 1987). In Australia, TBT antifouling is presently restricted to vessels greater than 25 metres in length, and in New Zealand there has been a complete ban on all TBT sales and use since December, 1993 (ANZECC, 1995). Internationally, interim measures have been recommended to restrict the use of organotins, and governments have been asked to consider the total prohibition of organotins in antifouling paints (ANZECC, 1995). No restrictions are presently enforced on copper-based antifouling, but it is highly toxic to many marine organisms, and alternative strategies are currently being reviewed by the Australian and New Zealand Environment and Conservation Council as part of its Strategy on Maritime Pollution.

Antifouling products for mariculture should be designed with a priority for minimising risk to non-target species (particularly those being cultured). In this regard, all chemicals intended for use in Australian aquaculture must be reviewed by a specific committee of the National Registration Authority. Lewis (1994b) recommended six criteria for antifouling strategies in the aquaculture industry. They should: (1) be effective against a broad range of fouling taxa, (2) be environmentally benign, (3) have no negative effects on the cultured species, (4) leave no residues in the cultured species, (5) be able to withstand on-shore handling and cleaning, (6) be economically viable.

One possible solution maybe the use of naturally occurring antifouling chemicals. Prior to use of modern polymeric netting, farmers in Malaysia have traditionally antifouled cotton nets by soaking them in tannins extracted from the bark of mangrove trees (*Rhizophora* sp.) (Lai *et al.*, 1993). However, whilst these absorb well to traditional fibre nets, the absorbancy to synthetic materials is poor and effectiveness is short-term

(Lai *et al.*, 1993). Although tannins act through toxicity, many marine antifouling chemicals act as chemical deterrents and deter fouling settlement at concentrations that are not toxic (Clare, 1996).

1.6 Summary

Biofouling of fish-cage netting is a significant operational problem to mariculture. The occlusion of mesh and the resulting restriction in water exchange, can adversely affect fish health by the reduction in DO and potentially by accumulation of metabolic ammonia. Fouling is of further concern because it significantly decreases cage flotation, increases structural fatigue and cage deformation, and may act as a reservoir for pathogens. The large surface area and structure of netting material, particularly multifilament mesh, is highly suitable for colonisation and growth of fouling. Furthermore, fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and excretory material) generated by the high-density fish populations. Consequently, the development of effective fouling control is particularly difficult, and is further hampered by the high species diversity and spatial variation typical of many fouling communities on cages. Whilst antifouling has been achieved with copper-based paints, the use of these products is undesirable because of possible environmental effects from metal-based toxins, and because of consumer concerns that may jeopardise market image. Consequently, most farms must conduct frequent changing and on-shore cleaning of nets. Alternative methods for fouling control, such as underwater cleaning or fouling-deterrent coatings based on naturally-occurring chemicals, are either in limited use or are undeveloped.

CHAPTER 2

In situ quantification of fish-cage fouling by underwater photography and image analysis

2.1 Introduction

Accurate, practical and reliable methods for fouling quantification are essential to provide meaningful comparisons of antifouling treatments, identify impacts on the performance of man-made structures, and supply ecological data on the development and dynamics of fouling communities. Techniques to quantify abundance, diversity and variation within fouling communities include destructive methods quantifying biomass and numbers of species (Bakus, 1988) and non-destructive methods to rate fouling severity (eg. Naval Ships' Technical Manual, 1989) and percent surface cover (eg. Butler & Connolly, 1996). Techniques to quantify a reduction in system performance include measurement of increased frictional resistance across surfaces (eg. Gucinski *et al.*, 1984), reduced heat-transfer efficiency from heat exchangers (Pandolfini & Avery, 1978), and reduced pumping efficiency through piping systems (eg. Rajagopal *et al.*, 1991).

Fouling on netting has previously been quantified by wet weights of test panels (*e.g.* Milne, 1975) or of communities removed from cages (*e.g.* Cheah & Chua, 1979; Lee *et al.*, 1985). However, the removal of fouling material from cages is destructive, time consuming and, given the shape and structure of netting, awkward. Alternatively, the occlusion of netting has been assessed either subjectively (*e.g.* Huguenin & Ansuini, 1981) or by the measurement of seawater exchange with a current meter (*e.g.* Gormican, 1989).

A requirement for all of these methods is adequate replication to overcome the high variability of fouling communities so that experiments have sufficient power to detect meaningful effects (Schoener & Greene, 1980; Schoener, 1984). However, logistic and economic constraints often limit replication in field trials, so appropriate sampling techniques must be chosen to extract the maximum information possible from a given experimental design. In Tasmanian waters significant differences in fouling have been found between different depths and sides of cages, and between adjacent cages (this study). Given this high degree of variability, considerable effort must be made to obtain sufficient samples to ensure accuracy. Sensitivity is also required for some trials, such as for the comparison of similar antifoulants or daily fouling growth.

The quantification of sessile aquatic macroorganisms has recently been conducted through image analysis (*e.g.* Ben-Zion *et al.*, 1991; Rahav *et al.*, 1991; Wright *et al.*, 1991; Haddad & Ormond, 1994). Haddad and Ormond (1994) used image analysis of photographs to monitor apical growth, lateral growth and change in total surface area of intertidal fucoid algae. Rahav *et al.* (1991) and Ben Zion *et al.* (1991) quantified the surface area and growth of the hydrocoral *Millepora dichotoma* from close-up underwater photography and subsequent image analysis. Wright *et al.* (1991) used image analysis to quantify tubeworm fouling on flat panels removed from the water, a method which resulted in substantial time-savings over manual counting techniques. They identified three critical factors for the successful use of computers to quantify fouling: sufficient contrast between substratum and fouling, homogeneity of colour or texture in the substratum, and the prevention of light reflection during image capture to increase contrast.

This chapter describes a method for image analysis of close-up underwater photographs of net fouling, to quantify fouling removal and regrowth in terms of mesh occlusion. Image analysis of photographs of net fouling enables large numbers of non-destructive samples to be taken and analysed quickly, compensating for the high degree of natural variability in the fouling communities. It also provides a permanent record of the fouling, allows dominant species to be identified and counted, and permits multiple sampling of the same area. As samples are recorded *in situ*, the reduction in mesh area can also be quantified and related to the reduction in water exchange.

2.2 Materials and Methods

2.2.1 Fouling Surveys

Trial 1: Temporal and spatial variation in mesh occlusion

A 65 m circumference cage (96 ply, 25 mm bar, cleaned black netting) was immersed on 1 November 1993, and mechanically cleaned *in situ* on 11, 17 and 24 November, and 3 December (*see Chapter 3*). Nine cable ties were placed at 6-bar intervals across sample areas at 1.0 m, 1.5 m and 2.0 m depth, to identify 27 sample sites for repeated observation (Fig. 3). Photographs were taken on 24 November (immediately after cleaning), 26 November, 1 December and 3 December (immediately after cleaning), using a Nikonos-V camera with an SB-103 strobe, 1:3 macro-extension tube and 100ASA film. However, on 24 November photographs were not taken at 2.0 m depth, and equipment failure on 3 December prevented cleaning at 2.0 m depth. To increase background contrast a black sheet was suspended behind the netting for photography on 1 and 3 December (Fig. 4).

In this trial, and in all subsequent trials, the background sheeting was placed upstream during photography. This shielded the netting from water flow so that occlusion measurements were not affected by variation in current rate and direction.

The rate of fouling growth was compared between each depth. A regression line was taken through the first three days of data for each depth and slopes of these regressions compared with the following formula:

$$t = \frac{(b_1 - b_2)}{S.E_{(b1 - b2)}}$$

b_1 = slope of line 1; b_2 = slope of line 2

where df = $(n_1 - 2) + (n_2 - 2)$ (Cohen & Fowler, 1990)

To quantify variability in the fouling communities and the precision of the photographic technique, the following protocols were adopted:

- On each sampling date, duplicate photographs were taken of each site.
- On 1 December photographs at 1.5 m depth were taken at 10.00 am and 1.00 pm to assess short-term changes in amphipod abundance.
- On 3 December the area adjacent to each sample site was photographed (Fig. 3) to test if the chosen sites were representative of each transect.
- At each depth and date the number and size of caprellid amphipods > 5 mm (from head to 7th pereonite) were recorded from the photographs.

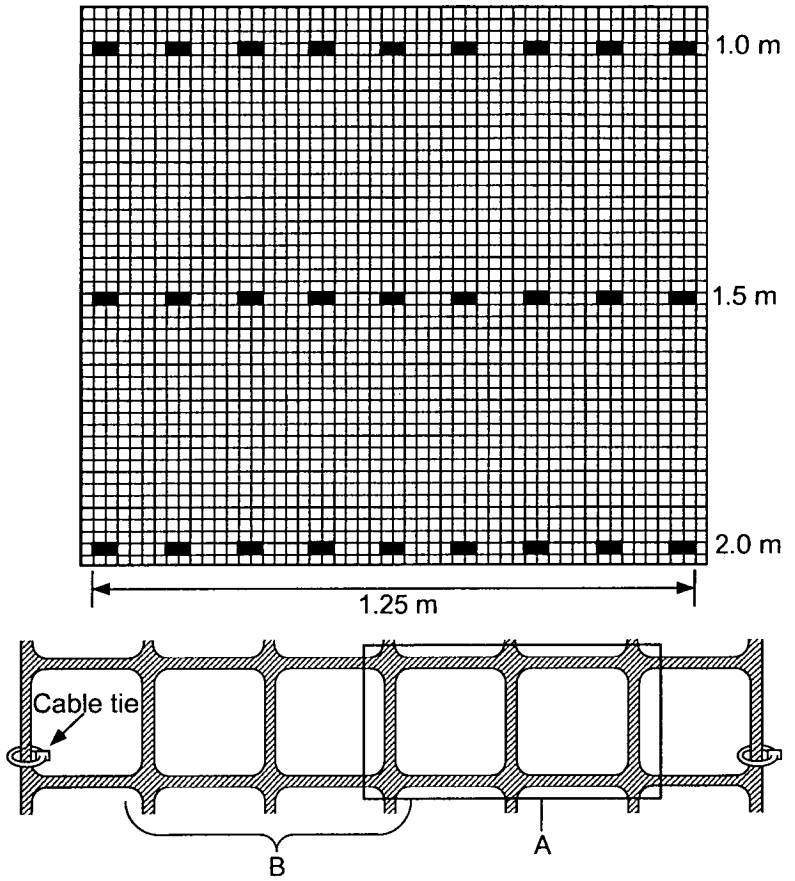
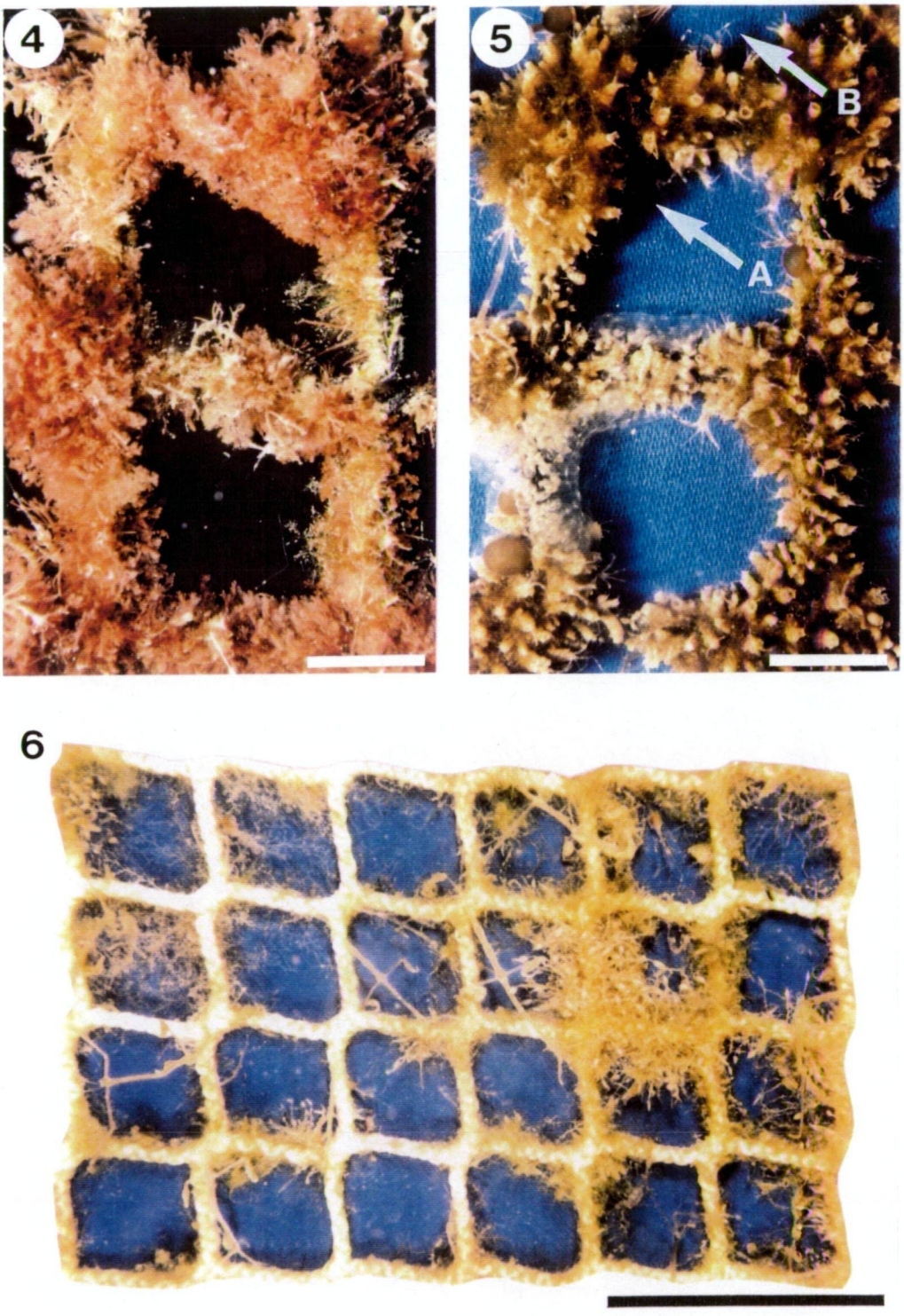


Figure 3. Nine cable ties were placed at 6-bar intervals across the sample area at 1.0, 1.5 and 2.0 m depth, to locate 27 sites for repeated observation. A = the area photographed per site covered 56 X 38 mm. B = the area adjacent to each site which was photographed on 3 December.

Trial 2: Temporal variation in mesh occlusion

A 5.4 m transect at 1 m depth was marked across the southern side of a 65 m circumference cage (96 ply, 25 mm bar, black netting). Across the transect (216 mesh holes) 36 pairs of non-overlapping mesh holes were chosen from a table of randomly-generated coordinates, and their location marked with a cable tie for repeated observation. Each pair was photographed on 29 April, and 5 and 11 May, 1994, using a Nikonos-V camera with an SB-103 strobe, 1:3 macro-extension tube and 100ASA film. The black background used in trial 1.1 proved difficult to distinguish from clean black netting (Fig. 4), so a blue sheet was suspended behind the netting for photography (Fig. 5).



Figures 4-6 *In situ* photographs of net fouling to be used for image analysis.
Fig. 4 Fouling growth dominated by the red alga *Antithamnion*, 7 d after cleaning. A black sheet suspended behind the netting increased background contrast with fouling. Bar = 1 cm. Fig. 5 A blue sheet behind the netting provided marked contrast with both the fouling and black netting, and was easy to recognise for image analysis. A = shadow caused by the use of only one strobe. B = blue material outside the 2 mesh holes in the sample area must be removed before image analysis. Bar = 1 cm. Fig. 6 Fouling left after cleaning during trial 1.4, photographed using two strobes. The blue material outside the sample area was cut from the photograph prior to scanning. Bar = 5 cm.

Duplicate photographs were taken at each sampling site. However, rather than taking the duplicate immediately after the first photograph (interval between duplicates < 10 seconds), two complete rolls of 36-exposure film were taken across the transect one-at-a-time, to create an interval between duplicates of approximately 60 minutes.

To identify which levels of the sampling design required the greatest replication (i.e. duplicate photographs or replicate sites) the data was analysed as a nested ANOVA using the statistical package JMP 3.01 (SAS Institute Inc.). The variance components for each level were then calculated from the *sum of squares* values according to Sokal and Rohlf (1995).

Trial 3: Spatial variation in mesh occlusion

A 16 m transect at 1 m depth was marked across the southern side of a 65 m circumference cage (96 ply, 25 mm bar, black netting). Photographs were taken on 9 September, 1994, after the netting had been immersed for 20 days. The transect was divided into 9 sub-areas, each containing 36 pairs of mesh holes. Twelve pairs of randomly chosen mesh holes were photographed in each sub-area. Photographs were taken using a Nikonos-V camera with two SB-103 strobes, 1:3 macro-extension tube and 100ASA film. The additional strobe was included to eliminate black shadows cast on the blue sheeting (Fig. 5, arrow A).

Trial 4: Fouling removal

A 65 m circumference cage (96 ply, 22 mm bar, new white netting) was immersed on 30 September, 1994, and mechanically cleaned *in situ* on 16 November and 14 December (*see chapter 3*). On the northern face of the cage 2 blocks were marked for sampling, one from 20-70 cm depth and one from 100-150 cm depth. Each block was divided into 2 replicate sections, each 90 mesh-holes wide (2 m) by 20 high. Photographs were taken on 13 December and 15 December to evaluate the efficacy of fouling removal by the cleaner. Both times 12 randomly-located sites (each 6 by 4 mesh holes, Fig. 6) in the sample sections were photographed twice. Photographs were taken using a Nikonos-V camera with two SB-103 strobes and 100ASA film. To increase the field of view (Fig. 6), a Nikonos close-up outfit (1:4.5) was substituted for the macro-frame.

2.2.2 Image analysis

Image capture and processing

Photographs were scanned into an IBM-pc computer (80486DX2-66) with an Epson® GT-6500 scanner and the software Epson Scan (V. 1.30). To identify clearly the mesh holes in the photographs from trial 1, the images were edited with Adobe Photoshop™ (V. 2.5.1) to give a distinct blue colour. To quantify mesh occlusion caused by amphipods (trial 1), a second version of each image was created with the amphipods removed. In trials where a blue background was used the edges were trimmed from each photograph prior to scanning (eg. Fig. 6) to remove blue material outside the sample area (Fig. 5, arrow B). Photographs from trial 2 were edited with Adobe Photoshop™ to remove black shadows cast on the background sheeting (eg. Fig. 5, arrow A).

Photographs taken with the macro-extension tube and close-up outfit represent cage areas of 56 mm X 38 mm, and 160 mm X 107 mm, respectively. The field of view for all photography was fixed by a frame attached to either the macro-tube or close-up outfit, and thus the absolute size of the sample could be determined. Photographs taken using the macro-tube (eg. Figs 4-5) were scanned at a resolution of 75 dots per inch (dpi). Due to the difference in scale, the photographs taken with the close-up kit (eg. Fig. 6) were scanned at 150 dpi to obtain comparable resolution.

Image analysis was conducted using IDRISI (V. 4.1) to determine the area (cm^2) of open mesh in each photograph. IDRISI is a geographic information and image-processing system developed at Clark University, Massachusetts, USA. Four IDRISI modules were used for fouling analysis. Images were first converted into IDRISI format (from 24-bit TIFF format) using the BIPIDRIS module. During the conversion process, images are separated into 3 colour bands so the module COMPOSIT was used to produce a 216-colour composite image. Each pixel in the image thus had a numeric value (from 0 to 215) which represented its colour. With the RECLASS module the value 9 (bright blue) was assigned to all areas in the image that contained values corresponding to the range of blues in the background. The AREA module was then used to measure the area covered by pixels with a value of 9. These 4 modules can all be used from the command line in MS-DOS®, and were included in 2 batch files (Table 2) so that they ran automatically, and repetitively, on the collection of scanned images. The measured value from each photograph was saved during processing.

Table 2. MS-DOS® batch files used to quantify the area of blue in scanned images.

GETIMAGE.BAT[a]

```
FOR %%A IN (*.TIF) DO CALL ANALYSIS %%A
```

ANALYSIS.BAT[b]

```
bipidris x %1 BAND 3 288 443 472 plane m 0 0.038 0 0.056 1
composit x BAND3 BAND2 BAND1 IMAGE 1 2 2.5
reclass x i IMAGE ONEBLUE 2 9 1 18 9 36 39 9 43 47 9 50 60 9 93 102 -9999
reclass x i ONEBLUE BLUE&BLK 2 0 18 36 0 39 43 0 47 50 0 60 93 0 102 999 -9999
area x BLUE&BLK 2 4 DATA
ECHO %1 >> ALLDATA
TYPE DATA.VAL >> ALLDATA
ECHO IMAGE NUMBER %1 COMPLETED
```

[a] file to repetitively run ANALYSIS.BAT on all images in the working directory
[b] file to run IDRISI commands for analysis of one image

Background contrast and shadows

The area of black shadowing cast on the blue background, and hence open area missed during image processing, was quantified to compare the use of one and two strobes. Background shadowing was quantified from:

- 30 photographs taken using 1 strobe and the macro-extension tube.
- 30 photographs taken using 2 strobes and the macro-extension tube.
- 30 photographs taken with 2 strobes and the close-up outfit.

Photographs taken with the macro-extension tube were dissected into two open mesh holes to remove black netting from the images. The netting photographed with the close-up outfit was white, so only the blue material surrounding the sample area was cut from the photograph (eg. Fig. 6).

2.3 Results

2.3.1 Fouling Surveys

Trial 1: Temporal and spatial variation in mesh occlusion

Image analysis demonstrated that fouling growth caused rapid blockage of netting over a 7-day period (Fig. 7). At 1 m the open area of the mesh decreased by 36% in 7 days, from 5.90 ± 0.07 cm^2 (± s.e) to 3.77 ± 0.18 cm^2 (± s.e.). The fouling community at each site was dominated by the red alga *Antithamnion* (Fig. 8), with significantly faster growth at 1 m compared with 1.5 m and 2.0 m depth ($P < 0.01$) (calculated from images excluding caprellids). Rapid growth of *Syncoryne* (Hydrozoa) colonies also occurred, but in lower abundance than *Antithamnion* at each site (Fig. 9).

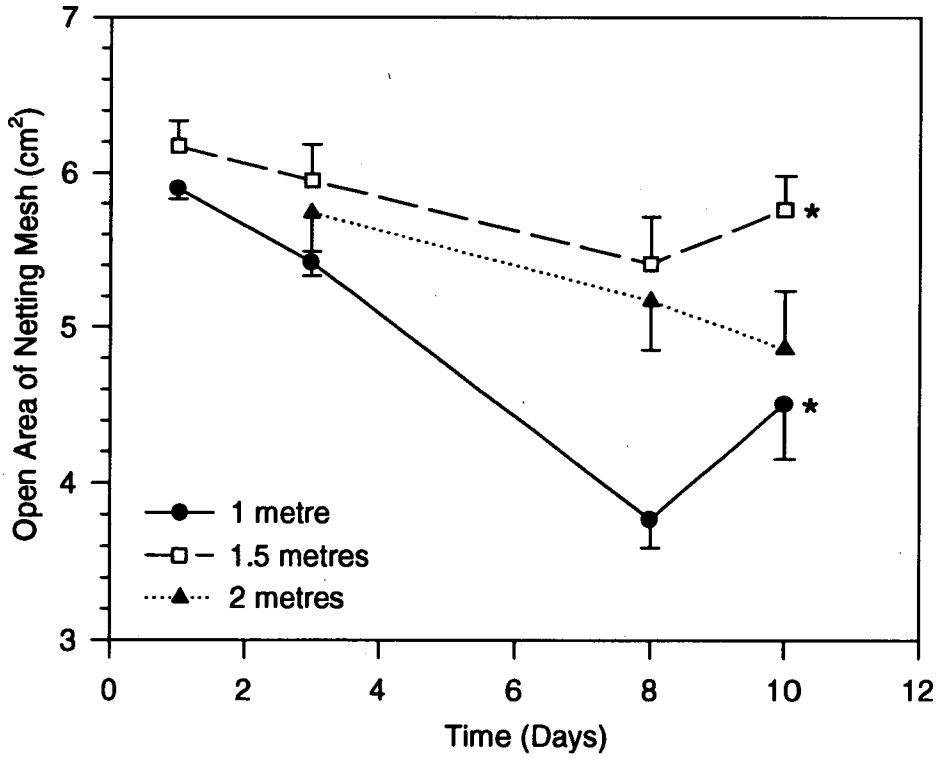


Figure 7. Mesh occlusion by fouling organisms (including caprellids). Each point represents the mean of 9 samples. * = after cleaning. Bars = standard error.

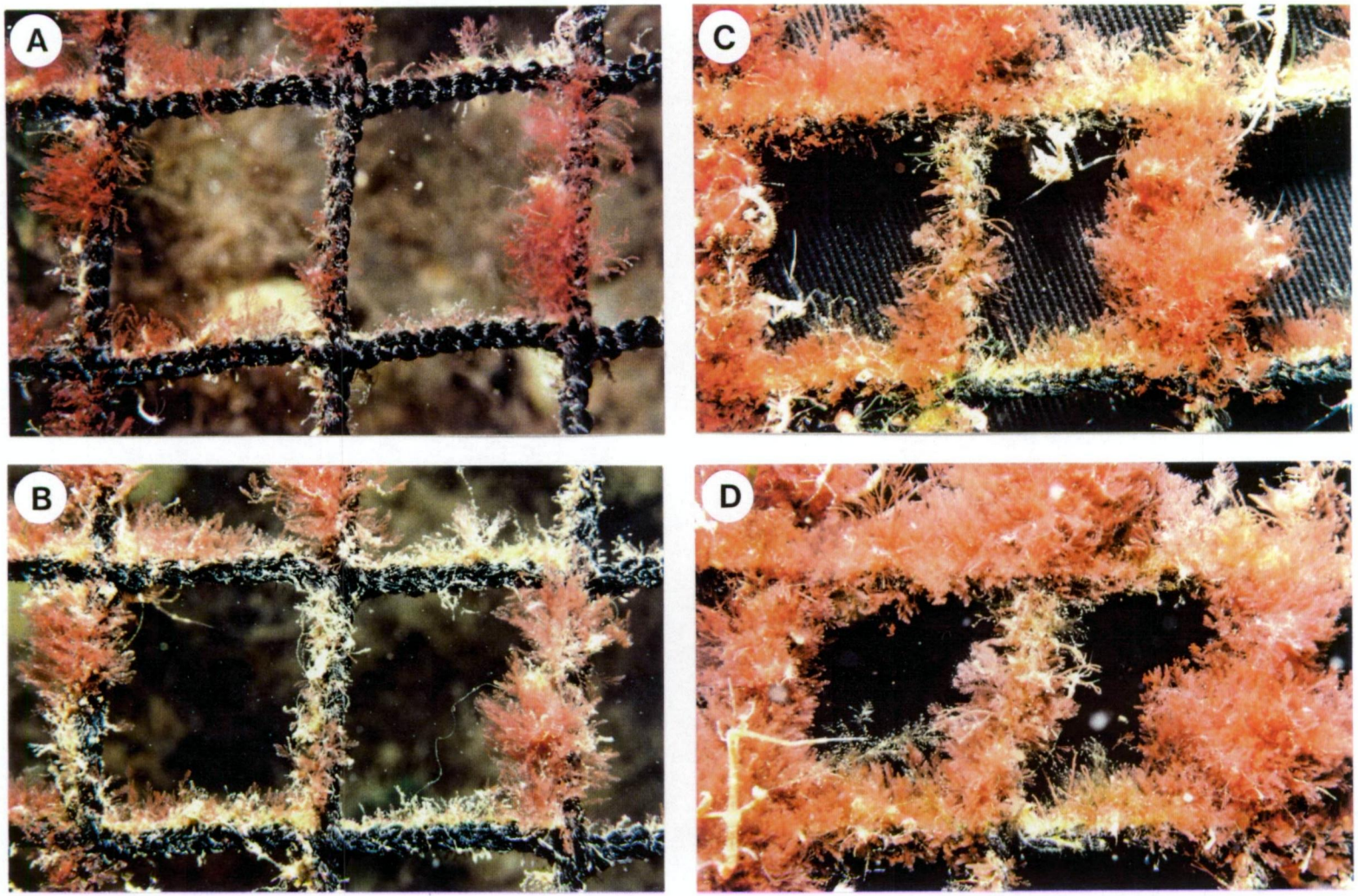


Figure 8. Fouling regrowth at 1 m depth, dominated by the red alga *Antithamnion*. A = immediately after cleaning. B = 3 days regrowth. C = 7 days regrowth. D = 10 days regrowth. The fouling community at this site was unaffected by cleaning (at 10 days) because of operating problems with the cleaner.

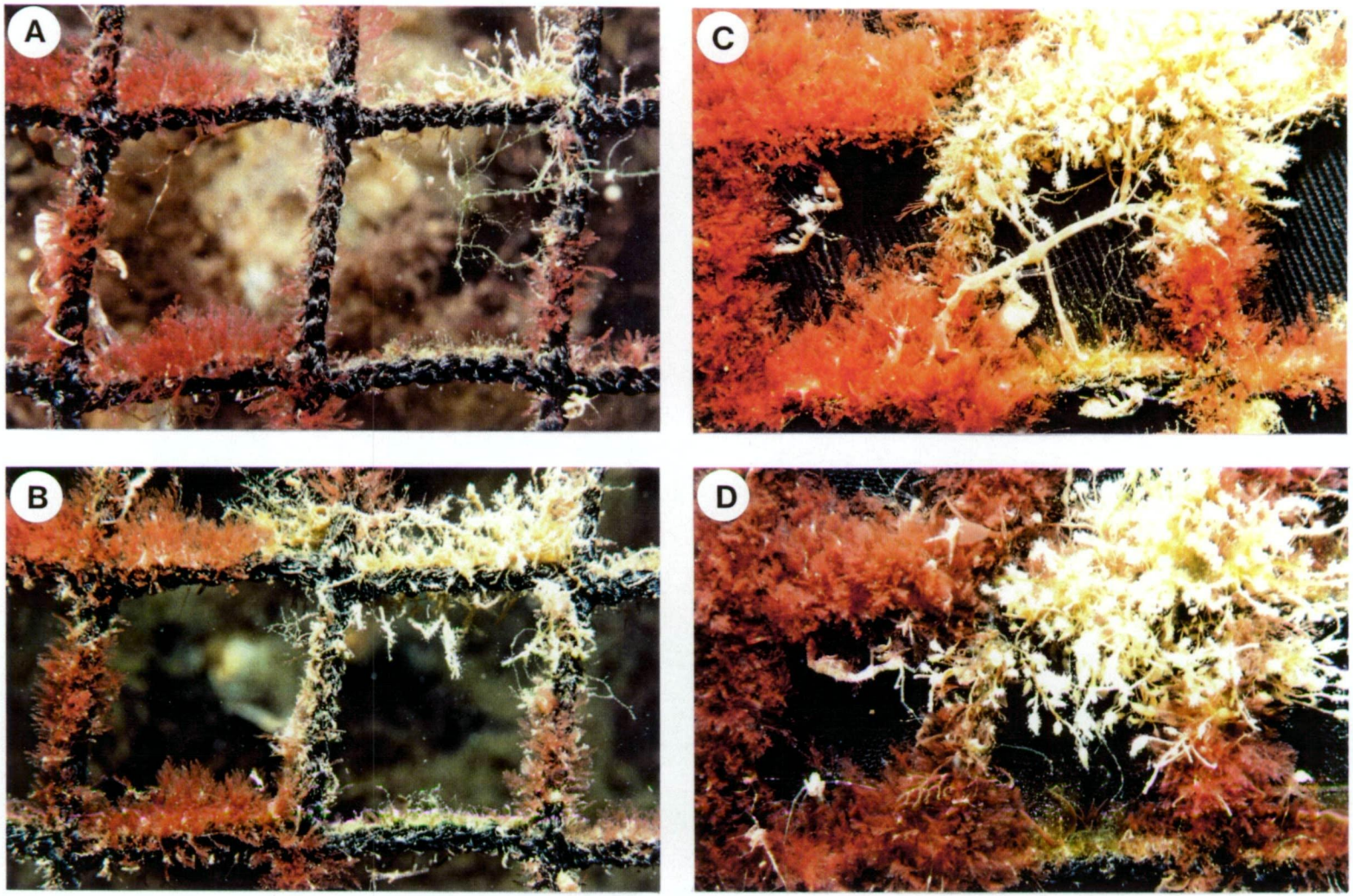


Figure 9. Fouling regrowth at 1 m depth, dominated by the red alga *Antithamnion* and the hydroid *Syncoryne*. A = immediately after cleaning. A = immediately after cleaning. B = 3 days regrowth. C = 7 days regrowth. D = 10 days regrowth. The fouling community at this site was unaffected by cleaning (at 10 days) because of operating problems with the cleaner.

Caprellid amphipods were typically a minor source of occlusion at each depth (Fig. 10), and reduced the total open area of netting by less than 4.0% (0.3 cm^2). The abundance of caprellid amphipods increased throughout the trial (Table 3), and caused a corresponding increase in mesh occlusion from this component of the fouling community (Fig. 10). Short-term changes in caprellid abundance caused large changes in mesh occlusion. On 1 December photographs taken from 1.5 m at 10.00 am (during a period of very high caprellid abundance, Table 3) showed caprellids to cause an 8.4% reduction in mesh area (Fig. 11). However, within 3 hours caprellid abundance reduced significantly (Table 3, Fig. 12), and caprellids caused only a 2.0% reduction in mesh area (Fig. 11).

Variation between duplicate photographs remained below 0.30 cm^2 (4% of the total mesh area) throughout the trial, but showed some increase with the presence of caprellids (Table 4). In comparison, variation between the 9 sites per depth was far greater and increased with fouling growth. At the time of greatest fouling (1 December) the standard deviations were 0.54 cm^2, 0.92 cm^2 and 0.95 cm^2 for 1.0 m, 1.5 m and 2.0 m depth, respectively. Notwithstanding this variability, the 9 sites sampled per depth (Fig. 3, A) were highly representative of each transect, and results were in good agreement with measurements taken adjacent to each site (Fig. 3, B; Table 5).

Table 3. Abundance and size range of caprellid amphipods on netting mesh.

		Size (mm), measured from head to 7th pereonite[a]					
Date	Depth	6 - 10	11 - 15	16 - 20	21 - 25	26 - 30	Total
24 Nov	1.0 m		1	4	1		6
	1.5 m	3					3
	1.0 m	2	5	3			10
26 Nov	1.5 m	2	6	2			10
	2.0 m	6	2		1	1	10
	1.0 m[b]	11	10	1	3	1	26
1 Dec	1.5 m (10.00 am)	9	20	8	4	3	44
	1.5 m (1.00 pm)	8	7	1	3	1	20
	2.0 m[b]	9	7	4		1	21

[a] Figures for each depth and date are the combined data for 9 sample sites.

[b] Photographs taken at 1.00 pm

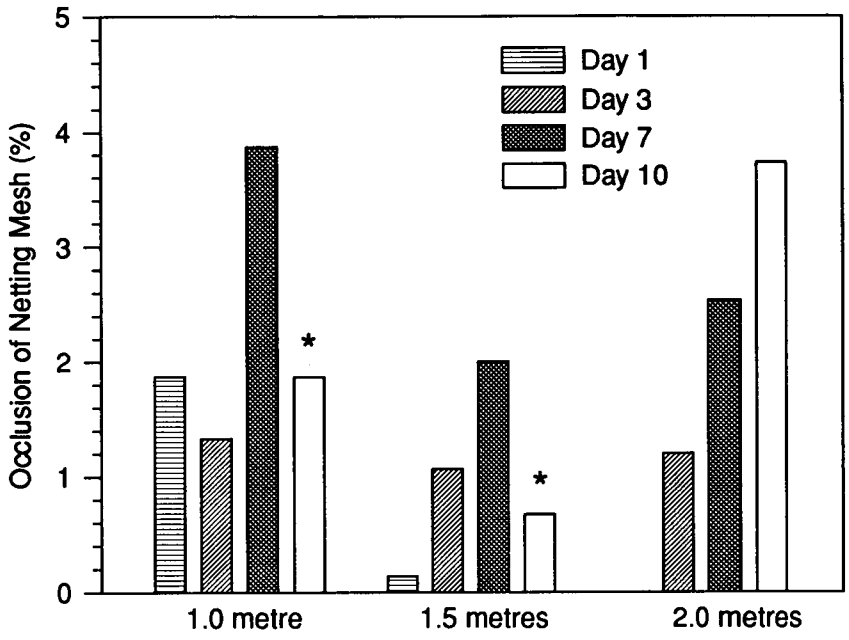


Figure 10. Mesh occlusion from caprellid amphipods. Each bar represents the difference between the mean open area per transect (n=9) and the mean open area per transect (n=9) determined when caprellids were edited from the scanned images. * = after cleaning.

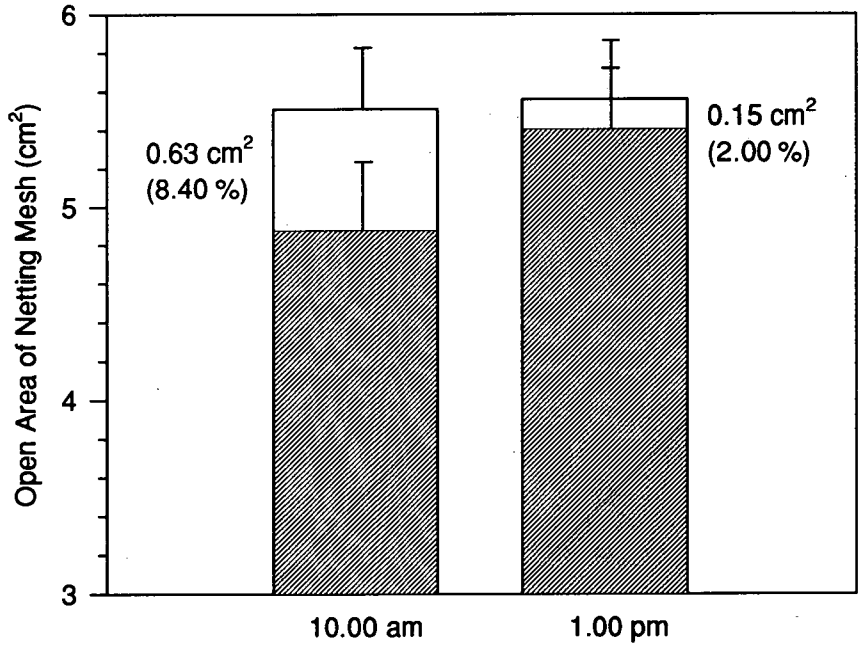


Figure 11. Short-term change in mesh occlusion caused by changes in caprellid abundance. Bars = standard error.

Mean open area (n=9) with caprellids edited from the images.

Mean open area (n=9) with caprellids included.

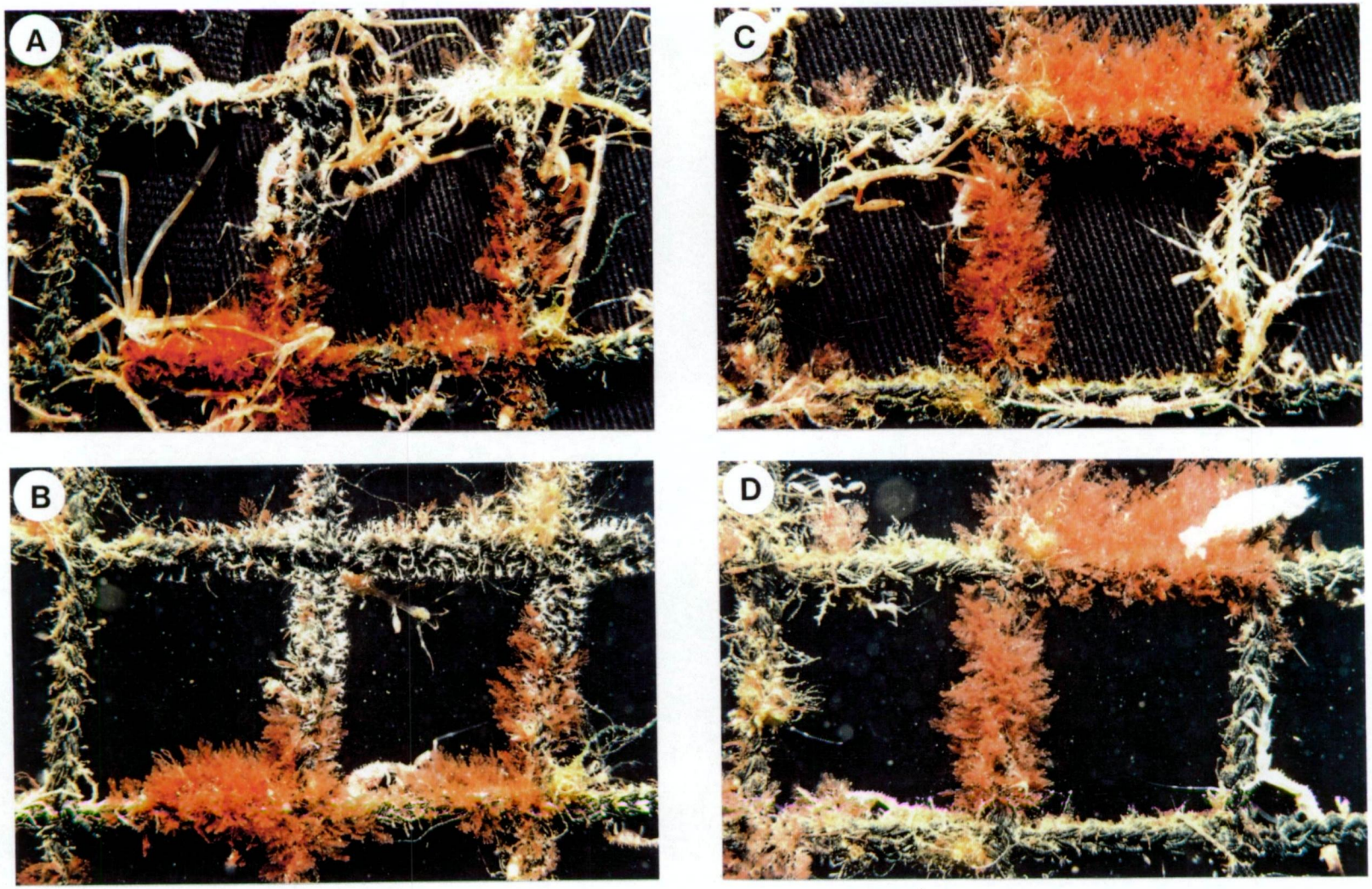


Figure 12. Short-term temporal changes in caprellid abundance. A = site 1, 10.00 am. B = site 1, 1.00 pm. C = site 2, 10.00 am. D = site 2, 1.00 pm.

Table 4. Mean differences between duplicate photographs on the 3 sampling dates, with and without occlusion from caprellids.

Date	Images including caprellids[a]	Images excluding caprellids[a]
24 Nov	0.27 ± 0.04	0.24 ± 0.04
26 Nov	0.15 ± 0.02	0.15 ± 0.02
1 Dec	0.21 ± 0.04	0.18 ± 0.02

[a] units = cm^2 ± standard error; n = 27

Table 5. Mean occlusion levels at original and adjacent sample sites on 3 December

Depth	Original sample sites[a]	Adjacent sample sites[a]
1 metre	4.51 ± 0.16	4.56 ± 0.17
1.5 metres	5.76 ± 0.09	6.08 ± 0.13
2.0 metres	4.86 ± 0.17	5.40 ± 0.34

[a] units = cm^2 ± standard error; n = 9

Trial 2: Temporal variation in mesh occlusion

Fouling growth at 1 m depth in May caused significant mesh occlusion within 13 days (Fig. 13), with a 36% decrease in mesh area from 6.67 ± 0.16 cm^2 (± s.e) to 4.26 ± 0.25 cm^2 (± s.e.). The fouling community was dominated by the green alga *Ulva rigida* (Fig. 14), with a small level of occlusion from caprellids and tube-dwelling amphipods (eg. Fig. 5).

Variation between duplicate photographs increased through the trial, with a mean difference between duplicates of 0.22 ± 0.04 cm^2 (± s.e), 0.44 ± 0.06 cm^2 (± s.e) and 0.52 ± 0.07 cm^2 (± s.e) for 1, 7 and 13 days, respectively. Variation between the 36 replicates also increased over time, with standard deviations of 0.95 cm^2, 1.17 cm^2 and 1.52 cm^2 for the means from 1, 7 and 13 days, respectively. The precision of the duplicate photographs relative to the variability between sampling sites was further demonstrated by nested ANOVA; the duplicates of each sites represented only 4.7% of the total variation, whereas the replicate sites accounted for 47.7%.

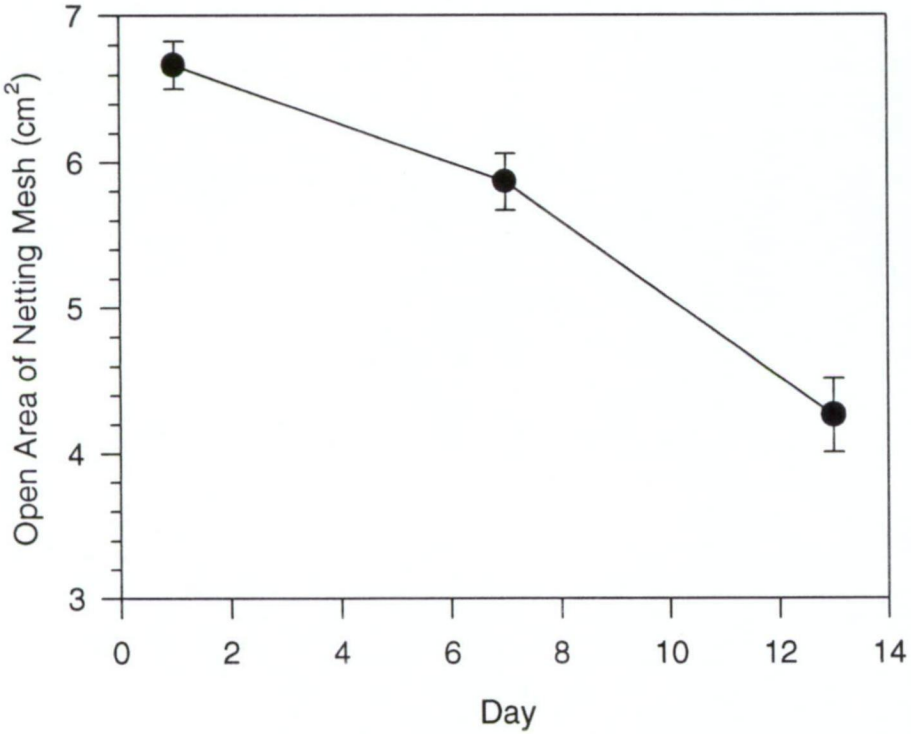


Figure 13. Changes in mesh occlusion across a 5.4 m transect at 1 m depth. Each point represents the mean of 36 samples. Bars = standard error.

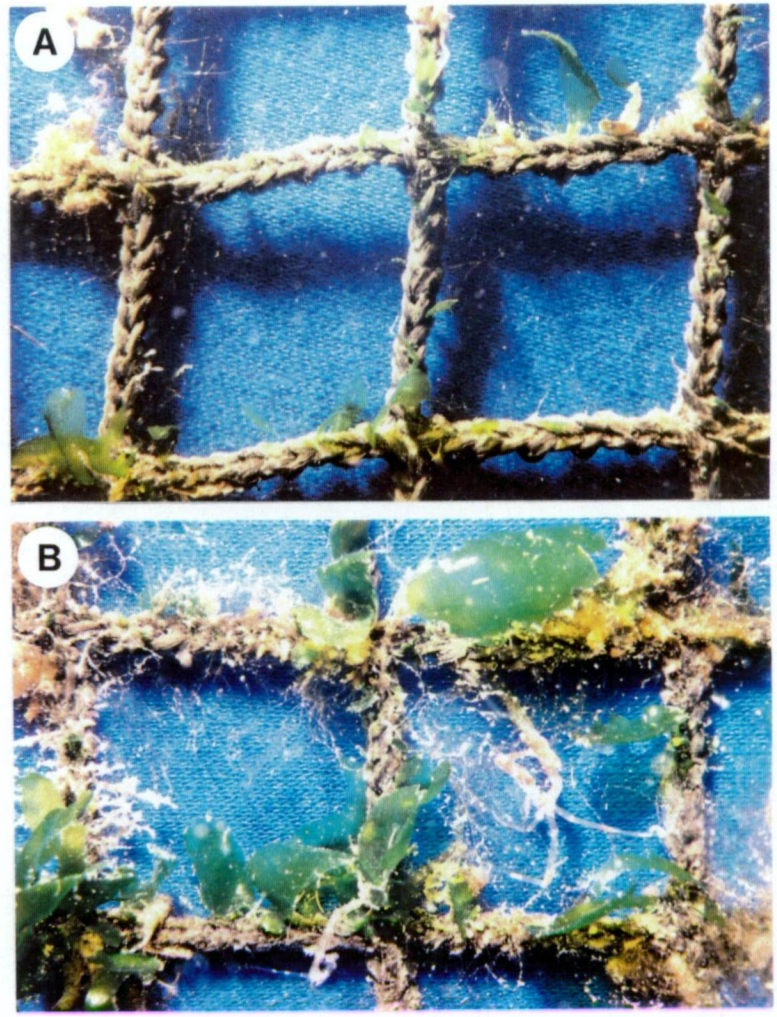


Figure 14. Fouling growth at 1 m depth in late Autumn, dominated by the alga *Ulva rigida*. A = day 1. B = day 13.

Trial 3: Spatial variation in mesh occlusion.

A high level of mesh occlusion was measured across the 16 m transect at 1 m depth (Fig. 15). There was no significant difference in mesh occlusion between the nine 1.8 m sample areas (P = 0.65, ANOVA), despite much variation in the composition of the fouling community (Fig. 16). However, because of the small difference in mean occlusion between the areas, and the high level of variation within each area (Fig. 15), the power of the comparison was only 0.33 ($\alpha = 0.05$).

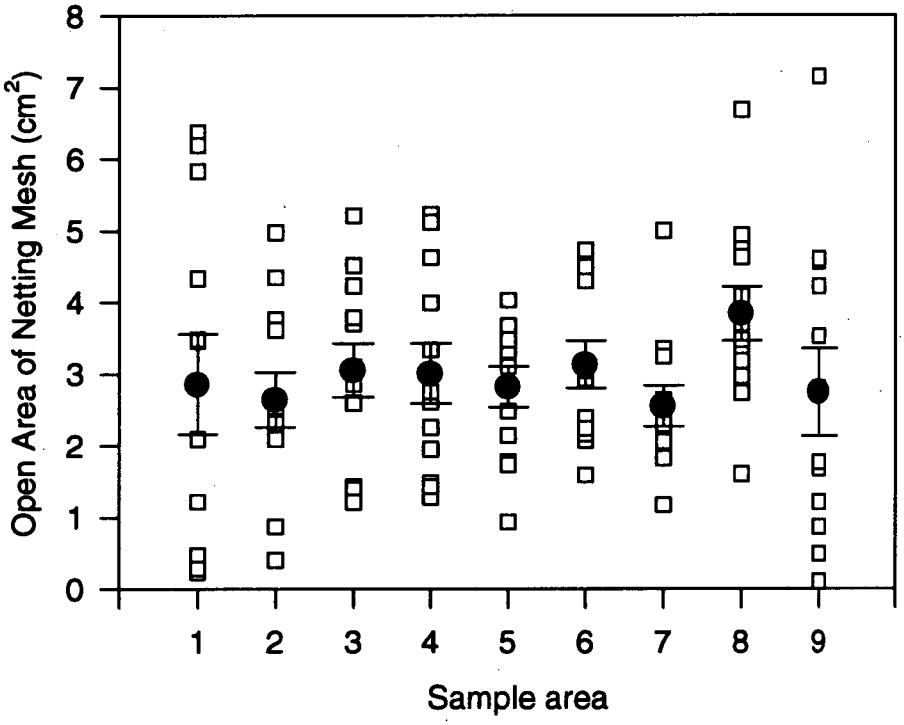


Figure 15. Levels of mesh occlusion across a 16 m transect at 1 m depth.
□ = individual sample site (2 mesh holes).
● = mean of 1.8 m sample area (n=9); bars = standard error.

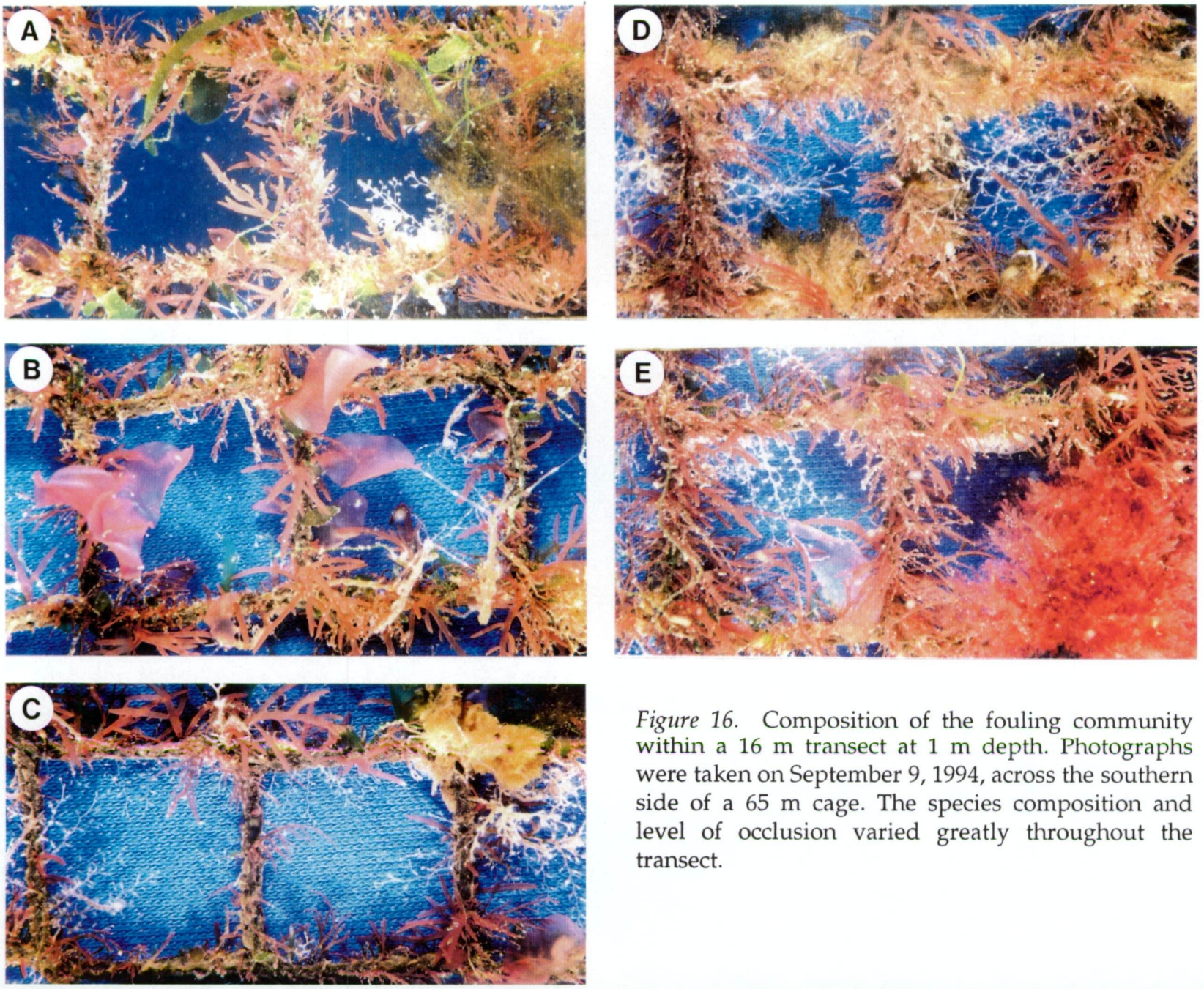


Figure 16. Composition of the fouling community within a 16 m transect at 1 m depth. Photographs were taken on September 9, 1994, across the southern side of a 65 m cage. The species composition and level of occlusion varied greatly throughout the transect.

Trial 4: Fouling removal

Analysis of the mesh occlusion levels (Fig. 17) found no significant difference in mean blockage before and after cleaning (P=0.75, ANOVA). Although the cleaner used in this trial (a different machine from that of trial 1) removed some fouling from the net during operation, contact between the cleaning brushes and the netting was insufficient to remove material close to the net. Consequently, the cleaner cropped the upper fraction of the fouling community, but left the lower structures unaltered, resulting in no reduction in net blockage. Significant differences were found between the two depths ($P < 0.001$, ANOVA) and between the replicate sections. The two sections in block 1 (20-70 cm depth) were not significantly different (P=0.16 before cleaning; P=0.87 after, t-test), however, there were significant differences ($P=9.6 \times 10^{-5}$ before; P=0.038 after) between the 2 sections in block 2 (100-150 cm depth).

The precision between duplicate photographs of each site remained high in all sections, with the mean difference between duplicates ranging from 0.81 ± 0.04 cm^2 (± s.e) (1.0% of total mesh area) to 2.7 ± 0.19 cm^2 (± s.e) (3.4%). The variance components for each level of sampling were calculated by analysing the four sections before cleaning as a nested ANOVA; this indicated that the duplicate photographs within each site contributed only 1.4% of the total variation, whereas the sites within each section accounted for 31.1%.

2.3.2 Image Analysis

Background contrast and shadows

Black shadows cast on the blue background were found to cause significant underestimation of open mesh area when only one strobe was used. Image analysis of photographs taken with the 1:3 macro-extension tube underestimated the mesh area by $16.6 \pm 0.9\%$ (± s.e.) when one strobe was used (eg. Fig. 5), but only by $3.0 \pm 0.7\%$ (± s.e.) when two strobes were used. Similarly, photographs from the close-up outfit (eg. Fig. 6) had minimal shadowing when two strobes were used, and mesh area was underestimated only by $2.7 \pm 0.8\%$ (± s.e.).

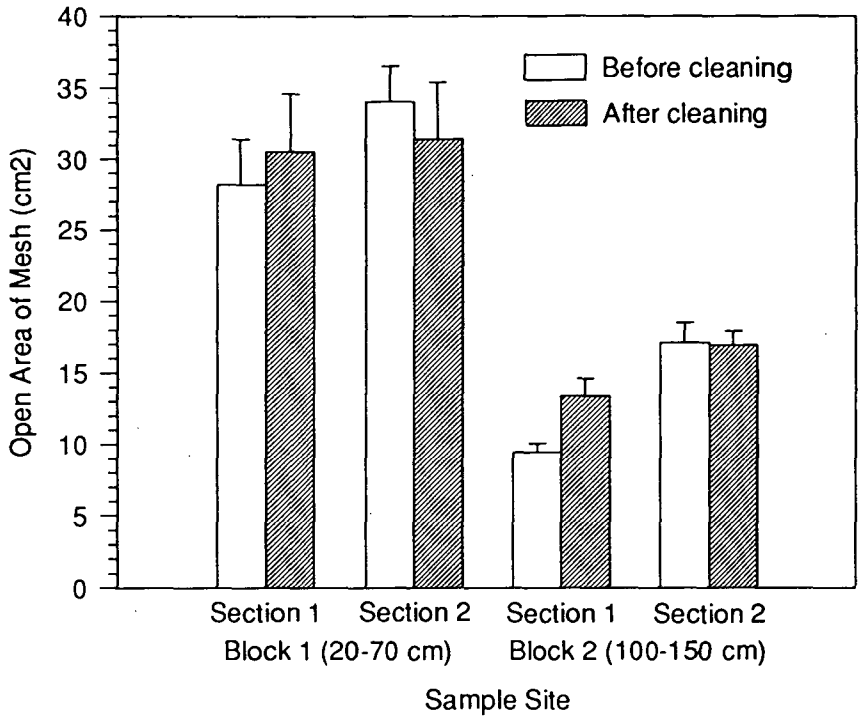


Figure 17. Fouling removal trial. The mean area (n = 12) of open mesh in each sample block, before and after cleaning. The total open area for clean netting is 80 cm^2. Bars = standard error.

2.4 Discussion

2.4.1 Fouling variation and sampling efficiency

The image analysis results demonstrate rapid fouling of fish-cage netting at 1.0 m depth, with a 36% reduction in mesh area over 7 days in summer (Fig. 7) and a 36% reduction in mesh area over 13 days in late autumn (Fig. 13). This fast development of occlusion is typical for Tasmanian waters, and nets must be changed every 5 to 8 days during summer (Hodson & Burke, 1994). A significant reduction in fouling growth from spring and summer to autumn and winter is also typical, due to large changes in water temperature, light availability and composition of the fouling community (eg. Figs 8, 14). However, the rate of fouling development remains high throughout most of the year and, as shown in trials 1 and 2, frequent sampling of fouling is necessary to adequately quantify temporal changes.

A significant depth effect was demonstrated in trial 1, with proliferation of algae at shallower depths (Figs 8, 9) causing a faster rate of occlusion at 1.0 m than at 1.5 m and 2.0 m. Similarly, a significant difference between depths was found in trial 4 where an abundance of algae at 100-150 cm caused greater occlusion than a hydroid-dominated community at 20-70 cm depth. Large changes over a small vertical range have also been reported in previous studies of cage fouling in shallow depths. Moring and Moring (1975) reported greater algal biomass at 0.5 m than at 1.0 m and 2.0 m, and Cronin (1995) found a greater biomass of *Ulva* at 1 m than at 2 m and 3 m. Vertical stratification primarily results from attenuation of light in the water column, but physical disturbance from wave action can also be important. Algae are often more abundant in exposed compared with sheltered environments because of a preference for sediment-free substrata (Shepherd & Womersley, 1970).

Small-scale spatial variation within a depth was demonstrated in trial 4, with a significant difference in occlusion between two adjacent 2 m wide sections. Large-scale variation between cage sides is well reported in the literature and is attributed to differences in exposure to direct sunlight (Moring & Moring, 1975). However, small-scale horizontal variation has not been studied on cages, despite the common observation of similar variation in other fouling communities (eg. Connell, 1975; Saenger *et al.*, 1979). This level of variation shows the need for relatively small sample areas to accurately measure fouling and detect changes in distribution. Furthermore, it demonstrates the need for a fast sampling technique that permits adequate replication of areas to compensate for the high degree of horizontal and vertical variation.

Variability in measurement of occlusion is increased by the movement of caprellid amphipods within sample areas (Figs 11-12). In trial 1 caprellids were found to cause < 4.0% occlusion of netting in the majority of samples (Fig. 10), except for one occasion where high caprellid abundance caused 8.4% occlusion at 1.5 m (Fig. 11). In this latter sample, a significant reduction in abundance from 10.00 am to 1.00 pm (Table 3), caused a 7.0% increase in open mesh area from 65.1% to 72.1% (Fig. 11). Movement also slightly increased variation between duplicate photographs (Table 4) because caprellids frequently react to strobe lighting. Consequently, when amphipods constitute a large component of net-fouling communities, the added variation to measurements must be considered in the sampling design.

The precision of occlusion measurements will be affected by movement of algae from changes in water flow. In trials 1, 2 and 4 duplicate photographs were taken of each site within a replicate area, and both pictures were used for image analysis. The open area (cm^2) for each site was then calculated as the mean of each pair of pictures. In each trial there was high precision between duplicates; in trial 1 the mean difference between duplicates was < 4.0% (of the total mesh area) for each transect and sampling date and in trial 4 the mean difference was < 3.4%. In trial 2 the variation between duplicates increased with fouling growth, with a mean difference of 2.9%, 5.9% and 6.9% for 1, 7 and 13 days, respectively. The lower precision in trial 2 probably reflects the reaction of different types of fouling to changes in water flow. The occlusion caused by dense tufts of *Antithamnion* (Fig. 8) and hydroid colonies (Figs 6, 9) is unlikely to vary greatly with minor changes in water movement, but the orientation of flat bladed algae such as *Ulva* (Fig 14) or *Porphyra* (Fig. 16, B) can change significantly.

In comparison with the duplicate photographs, a high degree of variation occurred between individual sampling sites within replicate areas (eg. Figs 15-16). The high precision of the duplicate photographs relative to the variation between sample sites was demonstrated by nested ANOVA. In trial 2 the duplicates represented only 4.7% of the total variation, whereas the replicate sites accounted for 47.7%. In trial 4 the duplicates contributed only 1.4% of the total variation, whereas the sites within each section accounted for 31.1%. The large variation observed among sample sites, but relative precision of the pairs of photographs, shows that resources would be better allocated by taking only one photograph per site and doubling the number of sites. This design improvement was confirmed by estimates of the expected variance of the means for two experimental designs in trial 4 (Sokal & Rohlf, 1981, p. 309); if 12 sites were sampled per

section and each photographed twice, the expected between-section variance is 4.6, however, if 24 sites are used and each photographed once the variance is only 2.4.

In trial 4, different sites were measured before and after cleaning. However, as the image analysis technique is non-destructive, the sensitivity of this type of trial could be improved by repeated measures sampling (eg. trials 1 and 2), rather than by increasing the number of sites. That is, the same 12 randomly-located sites could be photographed before and after the treatment and directly compared. This approach has some limits though, because repeated observations of fixed sites are not independent and there is some danger that sites with atypical fouling growth will be chosen. However, in communities where variation is low compared with the treatment (eg. growth), and a large proportion of the study area is sampled, atypical results are unlikely (eg. Table 5).

2.4.2 Fouling quantification using image analysis

Image analysis of underwater photographs is an easy and rapid method for quantifying the blockage of netting from the initial fouling growth on net cages. Four rolls of film (36 exposures) can be taken within 3 hours, thereby covering an area of 3064 cm^2 with a 1:3 macro frame, or 24,652 cm^2 with a 1:4.5 close-up outfit. The photographs from 1 roll of film can be scanned into the computer within 30 minutes. The image analysis required for 1 film took 1 hour for the 75 dpi images, and 4 hours for 150 dpi images, but, this processing requires no interaction from the user once the batch file (GETIMAGE.BAT) has been executed.

This technique can be difficult to apply with some types of fouling. Some compound ascidians are transparent and so cannot be recognised against the blue background (Fig. 5, lower mesh hole). Long filamentous forms of algae are difficult to photograph and assess for mesh occlusion as their form will vary greatly with different rates and direction of water flow.

Shadows cast on the background sheeting can cause significant error when only one strobe is used. Photographic trials demonstrated that the open area of mesh is underestimated by 16.6% ± 0.9 (± s.e.) when one strobe is used, but that this error is reduced to 3.0 ± 0.7% (± s.e.) for the macro-extension tube and 2.7 ± 0.8% (± s.e.) for the close-up outfit, when two strobes are used. Therefore shadowing is only a minor source of error when photographing with 2 strobes. Furthermore, so long as the shadow is a shade of blue (not black) then it will be correctly recognised as open area.

The application of image analysis to determine open areas in netting is simpler than its use to count fouling organisms on flat panels, as the organisms may overlap or have little contrast with the panel surface (Wright *et al.*, 1991). The ability to measure fouling *in situ* is advantageous, as repeated removal of the substrate from water may alter fouling communities (Schoener & Greene, 1981). A further benefit of the method is that photographs are taken close to the netting, which allows samples to be recorded in dark or turbid waters. Furthermore, the area of any colour in a photograph can be quantified with IDRISI, and thus the areas of different coloured fouling may be determined. The ability to sample fouling *in situ* and non-destructively, together with the creation of a permanent photographic record, combine to provide an excellent tool for net-fouling analysis.

CHAPTER 3

Biofouling of fish-cage netting: efficacy and problems on *in situ* cleaning

3.1 Introduction

3.1.1 The impact of biofouling - the Tasmanian Atlantic salmon industry

Marine farming of Atlantic salmon in Tasmania, Australia, has only developed over the past decade and is a relatively small industry by international standards. Atlantic salmon ova were successfully introduced to the state in 1984, and led to the first transfer of hatchery-reared smolt to sea cages in 1985 (Jungalwalla, 1991). The first harvest of salmon occurred in 1986 (53 tonnes), and the first salmon broodstock spawned in 1987 (Gjovik, 1987). Since then the industry has expanded rapidly, and hatchery production in 1996 was 1.6 million smolts (H. King, *pers comm*). The total harvest in the 1994/1995 financial year was 6,232 tonnes, worth $56 million (O'Sullivan & Kiley, 1996). The water temperatures in Tasmania (11°C to 18°C) allow salmon to be grown more rapidly than in Europe, and fish are marketed as a premium product due to an absence of many pathogens which makes chemical and antibiotic therapy unnecessary.

Cage use and design

Circular cages are almost exclusively used in this industry and are typically of 65 and 80 m circumference, and from 10 to 15 m in depth. At present there are approximately 250 cages in use, half of which are 80 m. Large cages are also being trialed, and there are currently seven 120 m cages and one 160 m cage in use. The salmon are stocked at 10-15 kg/m^3, with up to 50 tonnes in 80 m cages, 100 tonnes in 120 m cages and 200 tonnes in 160 m cages (Dietzel, 1996).

A large range of mesh sizes are used, with the smolt first stocked in 12 mm cages and gradually transferred to cages of increasing mesh size as they grow. Generally, mesh sizes of 12, 15, 17, 19, 25, 28, and 33 mm are used. Some farms also use 40 mm mesh, but this is less common because fish can become entangled.

The number of cages in use varies seasonally. The traditional production cycle begins with the transfer of smolt from the hatchery to marine farms in September and October (spring). At this time fish from the previous production cycle will be near harvest size, and will have been separated across a large number of cages due to grading. Consequently, during spring and summer (when fouling growth is most rapid) farms have

the greatest number of cages in use and also a large number of small mesh nets. Fish are harvested throughout spring and summer, so that there is a gradual decline in the number of stocked cages.

Over the past 4 years "out-of-season" smolt have also been produced (210,000 in 1995, H. King *pers comm*). These are transferred to the farms from April to May and thus fish are available for year-round harvesting (King, 1995). Consequently, farms now have a high rate of cage use throughout the year.

Changing and cleaning of nets

The rate of fouling growth varies seasonally, with significantly greater number of net changes required in spring and summer (Fig. 18). On average, nets are changed every 2 weeks during spring and summer, and every 5 weeks during winter and autumn. The smaller meshes are changed more frequently because their greater surface area makes them more susceptible to fouling and occlusion. During the period of peak fouling over 250 cages are in use, and therefore the industry, as a whole, must conduct approximately 500 net changes per month.

Net changing incurs a major cost to the industry, necessitating purchase of a large number of nets and provision of dedicated net-changing and cleaning teams. During changing, the fouled net is partially raised and a clean net is peeled underneath and attached to the collar. The fouled net is then untied and removed, with the fish released into the clean cage. Boat-mounted hydraulic cranes are required to remove the nets because fouling causes a two- to three-fold increase in weight; fouled 80 m nets often weigh more than 2 tonnes. Fouled netting is left to compost for 1-2 weeks on-shore, followed by cleaning with automated washing machines (Fig. 19). The cost for net removal, replacement, cleaning and maintenance is currently estimated at $550 per net change (J. Smith, TASSAL, *pers comm.*), and therefore the total cost across the industry is geater than $250,000 per month during spring and summer.

Net washing and on shore-handling procedures (typically with fork-lift trucks) cause significant wear and damage to the nets, and reduce their useful life-span. After cleaning the nets are laid out for mending and replacement of damaged sections. However, weakened sections can be missed during inspection which risks the potential loss of stock during future use. The time period required for washing, drying and repairing also means that large numbers of nets must be purchased as many will be out of service at any one time.

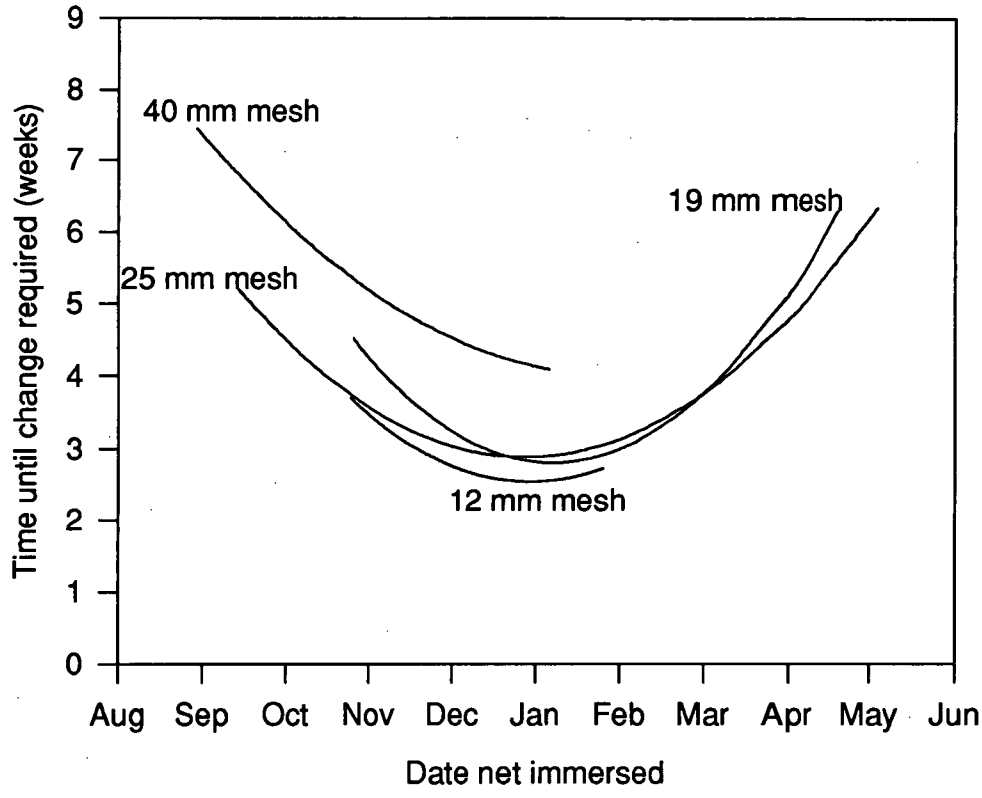


Figure 18. Frequency of net changing at an Atlantic salmon farm in Tasmania (data collated from farm records supplied by Aquatas Pty. Ltd.).

The majority of Tasmanian farms use anti-predator netting to prevent cage damage and loss of stock from seal predation. Commonly, each cage is contained within a larger mesh (150 - 200 mm) net, although at some sites a large section of the farm is enclosed with a predator fence. The high cost of maintenance (primarily fouling removal) associated with the latter strategy has seen the fencing system recently dismantled at one farm. A large fence comprised of 18 x 50 metre sections to enclose a 12 ha area is currently used at one farm. At its deepest point the fence extends to 30 m, and is also divided vertically for ease of maintenance. The upper area of each section (5 metres deep) is replaced for cleaning at least three times per year, and the lower area twice per year. The annual cost for maintenance of this perimeter fence exceeds $50,000. Although the large size of anti-predator netting allows longer immersion periods before fouling is a problem, the changing and cleaning of nets and fences is a further significant cost to the industry.

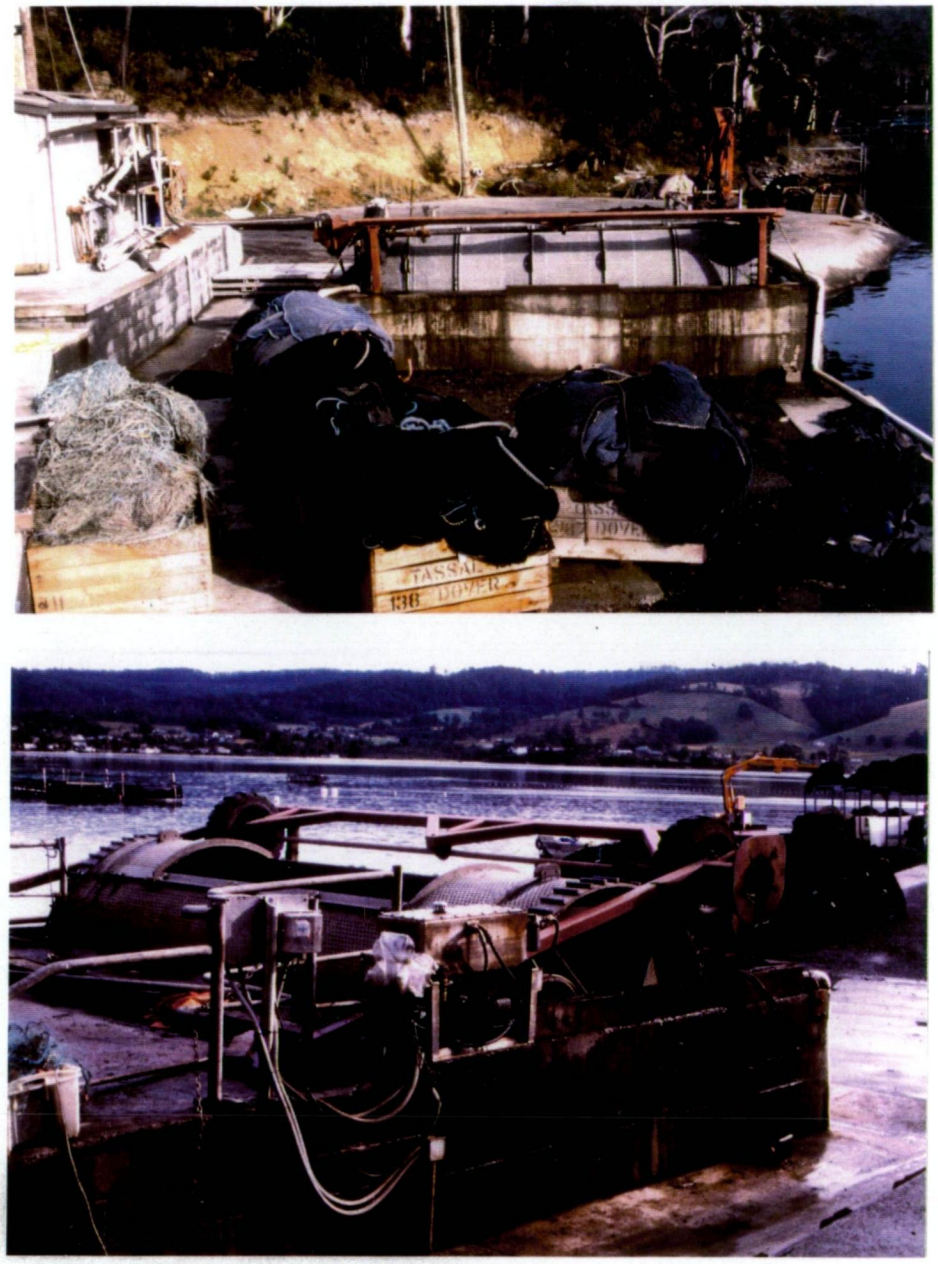


Figure 19. Conventional on-shore net washer, comprised of a large rotating cylinder partially immersed in a freshwater bath. Nets are left to compost on shore for 1-2 weeks prior to cleaning. After cleaning nets are mended to repair damage caused by cleaning and on-shore handling.

The cost of biofouling to farm production and efficiency

Frequent net changing risks damage or loss of stock, and disturbs feeding regimes which lowers growth rates. The frequent changing of 12 mm mesh during spring and summer is an added stress to smolt which are going through a period of saltwater acclimation. Furthermore, the fish are under increased stress during this period because water temperatures are at the upper limit for Atlantic salmon culture and amoebic gill disease is prevalent throughout the industry. The latter increases the need to handle fish because every 6-12 weeks the fish must be temporarily bathed in fresh water to remove amoebae (Howard & Carson, 1995). Whilst it is desirable to reduce handling stress by minimising the number of net changes, the need to ensure unrestricted flow of oxygenated water into the cages is utmost during the warmer months. The increased temperature causes increased respiration rates in the fish and a decrease in oxygen solubility in the water (Lagler *et al.*, 1977; Meadows & Campbell, 1978).

The crowding of fish that occurs with partial raising of the fouled net during replacement, increases the risk of abrasion and exposure to pathogens. For example, the combination of handling stress and physical damage increases the susceptibility of salmon to erosive skin disease caused by *Flexibacter maritimus* (Munday, 1996).

Handling is also commonly observed to cause a loss in appetite and fish will not feed for at least half a day after a net change. With each fish experiencing at least 30 net changes, more than 15 days of feeding are lost over a production cycle.

Fouling prevention

Fouling protection for fish cages has partly been achieved through use of toxic, copper-based antifoulants (Lewis, 1994b). However, the use of these products is undesirable because of possible environmental effects from metal-based toxins, and consumer concerns that may jeopardise market image. The latter is of particular concern with Tasmania, because the low incidence of disease, a ban on antibiotics, and the location of farms in pristine waters are used to market the salmon as a premium, high-quality product. At present no antifouling is used in this industry.

Antifouling treatments are also ineffective against masses of drifting algae which become entangled in netting. For example, in summer in Tasmania large masses of *Enteromorpha paradoxa* commonly become entangled in nets, with 2-5 metres of the thallus extending into the water and thus away from antifoulants.

3.1.2 Underwater cleaning

General techniques

All current antifouling systems have a limited life-span, and are eventually colonised by a variety of micro- and macroorganisms. Biofouling problems, and the expense or impracticality of removing fouled surfaces from water have led to the development of underwater cleaning technology for oil platforms (Pearce, 1994) and ship hulls (Alberte *et al.*, 1992).

A large range of equipment has been successfully used to dislodge fouling, including explosives (Gracey, 1974), cavitating water jets (Conn, 1980), and serrated rotating cutters (Pearce, 1994). However, the most common methods are based on surface scrubbing with rotating brushes. On large ships, sets of rotating brushes have been held against the hull by an impeller and guided along the ship from a small boat (NSTM, 1989). On smaller ships diver-held rotary brushes and semi-automatic diver-guided multibrush vehicles have been used (Cologer *et al.*, 1977). Piping and heat-exchanger tubes have been successfully cleaned by scrubbing with flow-driven brushes (Nickels *et al.*, 1981). On oil platforms floating brush units are fitted to vertical tubular sections and the surfaces are cleaned as the units move up and down from wave action (Pearce, 1994).

The effects of underwater scrubbing on biofouling communities have been described for ship hulls (Moss and Marsland, 1976a) and the internal surfaces of seawater-cooled heat exchanger tubes (Nickels *et al.*, 1981). Both groups reported that the cleaning process selected for a fouling community which regrew faster following repeated cleaning. Moss and Marsland (1976a) observed that the majority of algae was effectively removed by scrubbing, but small quantities of rhizoids and basal parts of filaments remained, particularly in crevices. Remnants of *Enteromorpha* cut off just above the basal rhizoids were able to regenerate into a new plant with many new branches. The problem of regrowth was thought to be further exacerbated because scrubbing fragmented reproductive cells, which released vast quantities of swarmers that can immediately recolonise the surface.

At present *in situ* cleaning of mariculture cages is not common practice, and is complicated by the 3-dimensional structure of netting, the many differences in cage design and the necessity of removing debris from the water column.

Development of *in situ* cleaning equipment for the Tasmanian salmon industry

In 1992 an industry workshop was held to discuss potential methods of fouling-control for salmon cages in Tasmania. Development of a device for *in situ* mechanical cleaning of nets was considered to be of highest priority and an industry working group was formed to compile a research proposal (Doedens, 1992). Three methods of net cleaning were considered (suction, water-blasting and scrubbing), with scrubbing identified as the most practical and efficient option (Lewis, 1993). The project then proceeded in two stages with funding provided by the salmon industry and the state government (Lewis, 1994c). In 1993 a diver-operated, hydraulic brush-test unit was constructed to allow preliminary studies of the efficacy of cleaning nets with rotating brushes. From this work the design parameters for a large-scale prototype were identified (i.e. bristle types, length, strike rate), and in 1994 an automated cleaner that could track around the circumference of a cage was constructed.

The following chapter describes the series of trials used to determine the effectiveness of the hydraulic brush-test unit and the automated prototype cleaner. The removal of fouling, and the problems of residual structures, resistant communities and accelerated regrowth are described and discussed. The results demonstrate many difficulties that may limit the efficacy of *in situ* cleaning, but suggest that this technology can significantly extend the immersion time of nets during seasons of peak fouling.

3.2 Description of the field site

Trials were located at Saltas Marine Operations in Port Esperance (43°20' S, 147°01'E), Tasmania, Australia (Fig. 20). The farm is situated in an estuary where water movement is dominated by tidal flow (mean current velocity < 2 cm/sec), and cages are moored in approximately 20 m of water. The waters are fully marine except for a 2 - 8 ‰ drop in salinity to 0.5 m depth after high rainfall in winter. Water temperature from November through January ranges from 13 to 17°C at 1 m depth, and is generally 1 to 2°C cooler below this. The total farm biomass and daily feed rate throughout the trial period are shown in figure 21. The two trial cages for testing of the prototype cleaner were located in a block of 8 cages containing a total biomass of 48.50 t on October 31 and 43.69 t on February 10. The trial cages were not stocked due to concerns that debris from cleaning may affect fish.

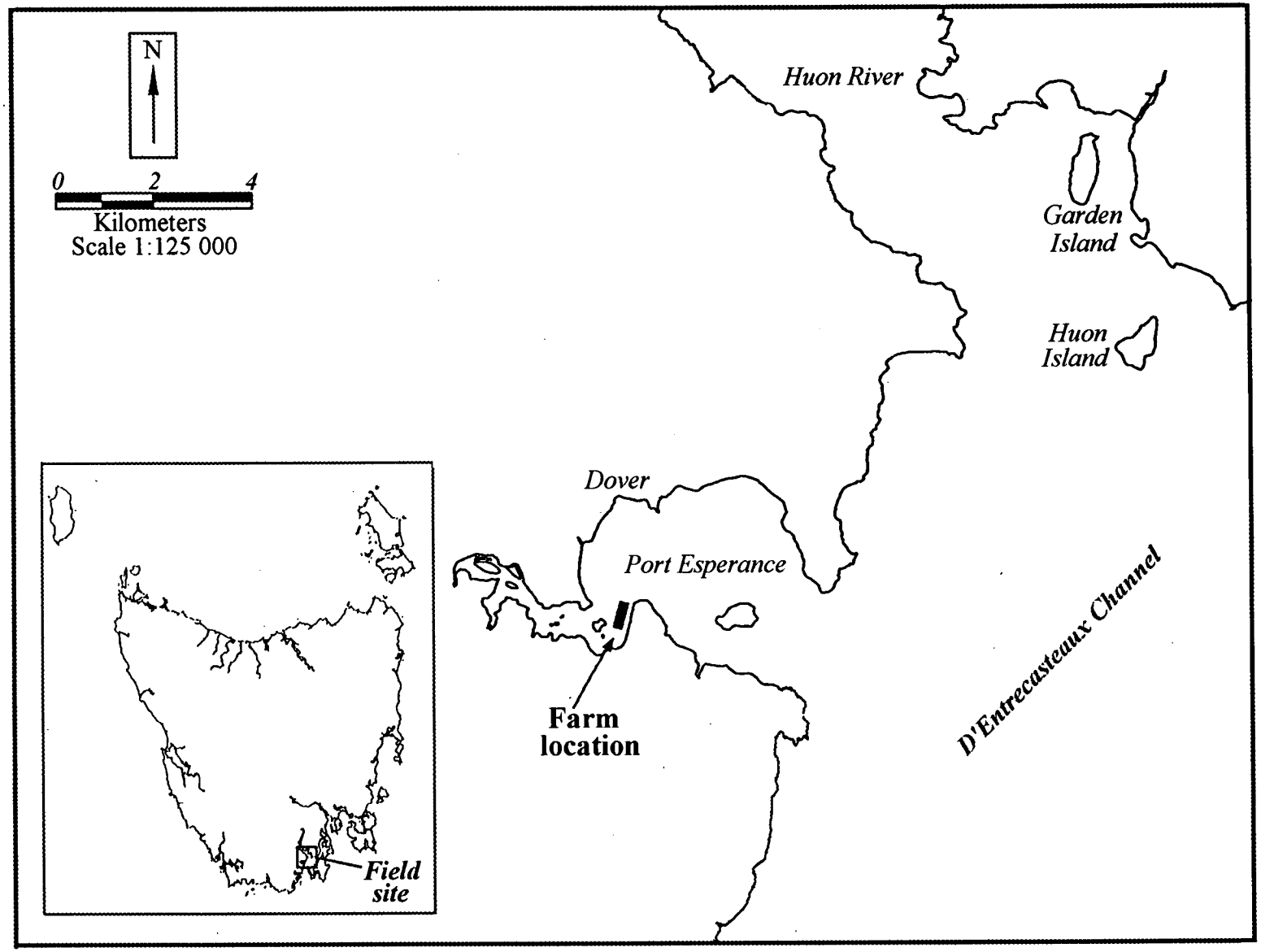


Figure 20. Location of field site for trials of *in situ* cleaning.

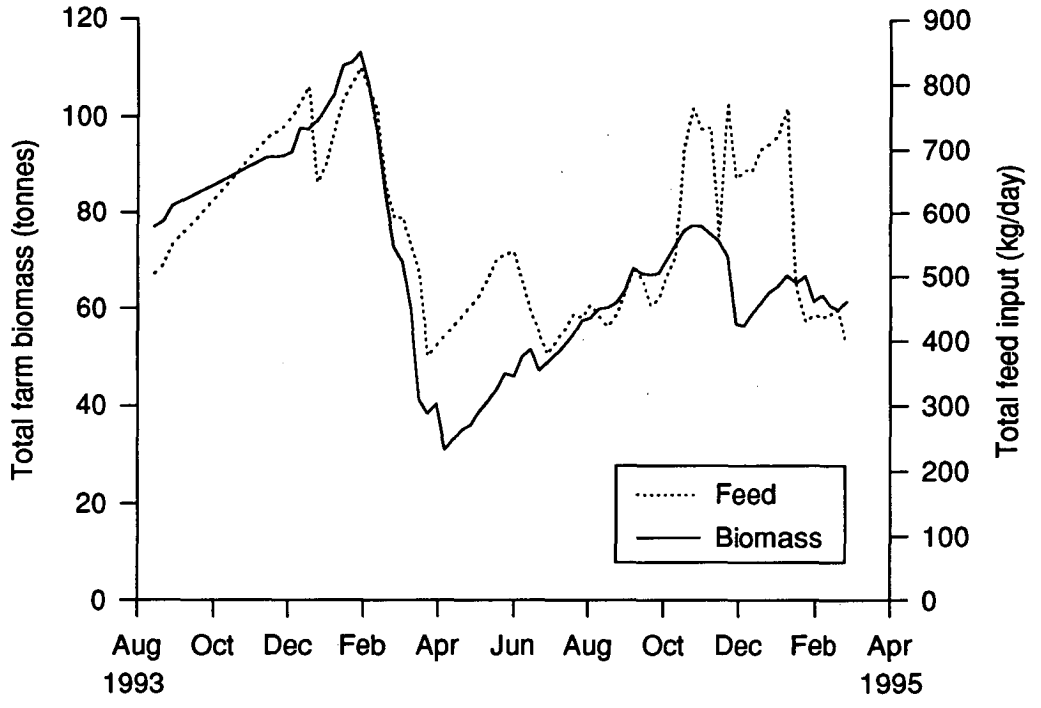


Figure 21. Farm biomass and daily feed input during underwater net cleaning trials.

3.3 Evaluation of the brush-test unit

3.3.1 Materials & Methods

Unit design

The brush-test unit was configured with one centrally-mounted cylindrical brush (Fig. 22), and designed to allow easy interchange of different brushes. Six brushes were built, and provided a comparison of three bristle types ("hard", "medium" and "soft") and two lengths for each type (45 mm and 25 mm). The unit was diver operated and driven by a petrol-powered hydraulic unit (boat mounted) with enough hydraulic hosing for operation to 4 m depth.

Hydroid removal

The brush-test unit was used on the eastern side of a 65 m cage (96 ply, 25 mm bar) on August 4, 1993. The cage had been immersed for 2 months and on the eastern face was heavily fouled with the hydroid *Obelia australis*. This fouling community was specifically chosen because hydroids have been reported to be more difficult to remove from netting than algae (Huse *et al.*, 1990).

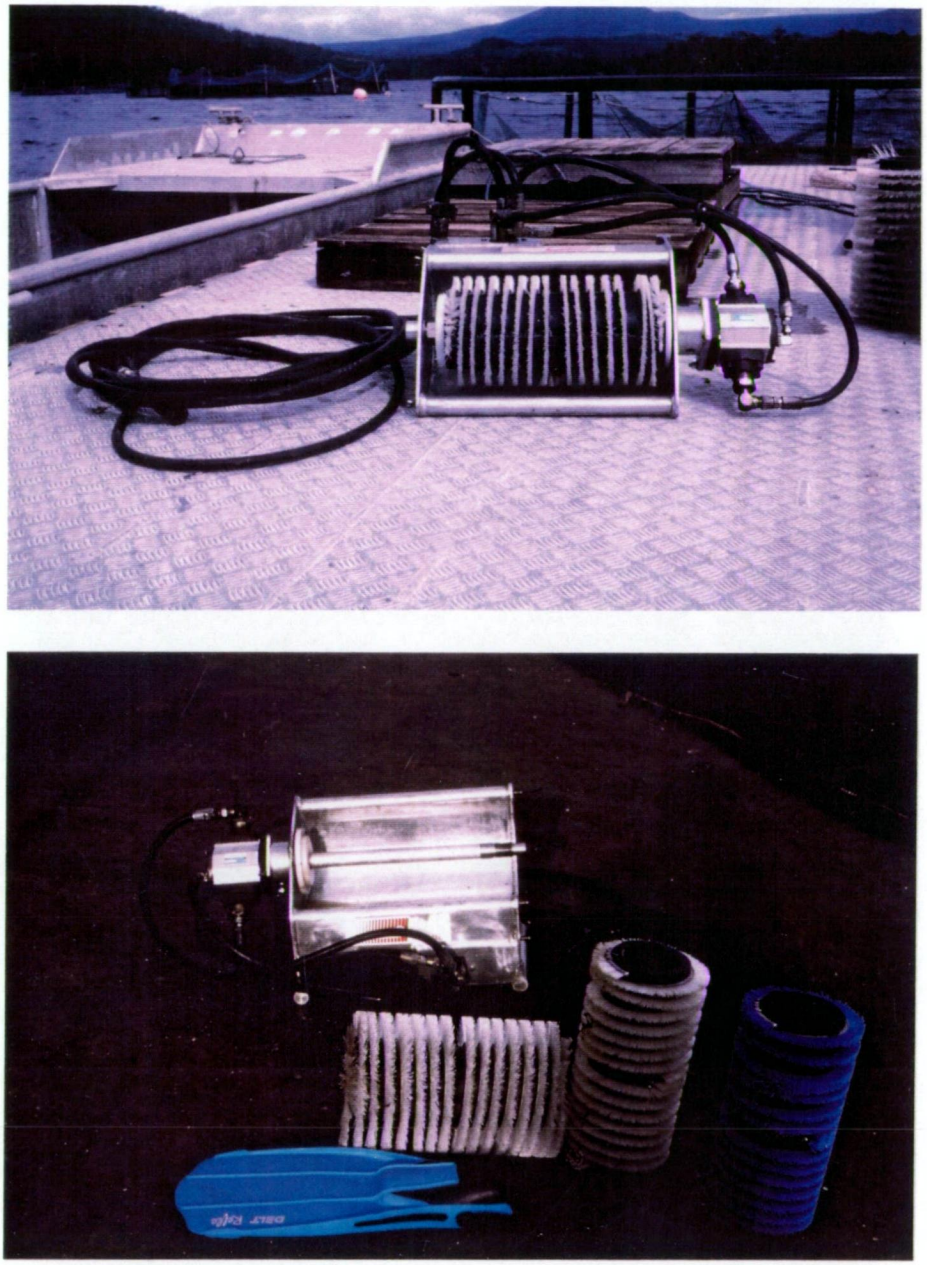


Figure 22. The brush-test unit used to determine the efficacy of *in situ* cleaning with brushes and to identify the most suitable type of brush for cleaning. The centrally mounted brush could be removed easily for interchange with different brush types.

Operation of the unit and collection of samples was conducted using SCUBA. The cleaner was operated by one diver at 0.5 to 1.0 m depth whilst a second diver, at approximately 4 m depth, held the net taut. The trial was conducted on the inside surface of the cage with the cleaner held in a horizontal position and the brush rotating downward.

Both hard and soft bristles (both of 25 mm length) were tested, with 8 samples (25 mm bars) of cleaned netting collected for each. Samples of netting were cut from the cage at 30 cm intervals across the cleaned areas and immediately placed into plastic sample tubes. Samples were fixed for scanning electron microscopy (SEM) within 1 hour, and prepared according to Hodson and Burke (1994).

Damage to netting

For each of three brush types (25 mm soft, 25 mm hard, 45 mm soft) the brush-test unit was fixed to a 600 mm x 750 mm aluminium frame that contained new 96 ply, 25 mm bar netting. The frame and unit were immersed over the side of a boat and the netting scrubbed for 5 minutes. Macro photographs were taken to record the condition of each net.

Fouling removal and regeneration

A 65 m circumference cage (96 ply, 25 mm bar, cleaned black netting) was immersed on 1 November 1993, and mechanically cleaned *in situ* on November 11, 17 and 24, and December 3. Fouling removal and regrowth was quantified as described in chapter 2 (*Temporal and spatial variation in mesh occlusion,* Fig. 3). To provide a comparison between the cleaned area and the remainder of the cage a 1.25 m transect adjacent to the cleaned area was sampled at 1 m depth on 3 December. Within this area 9 sites at 6-bar intervals were photographed.

To create a taut surface during cleaning the cage was temporarily tied to the predator net. The brush-test unit was operated with the brush in a vertical position (45 mm bristles, softest type), and the area cleaned by making horizontal sweeps at increasing depths.

3.3.2 Results

Hydroid removal

The cleaner was highly efficient at removing hydroids from those areas which were in direct contact with the brushes. Visible fouling was removed from the inner surface of the cage, and SEM demonstrated that microalgae and protista were also removed (Figs 23, 24). However, significant fouling remained on the outer and lower surfaces of the bars (Figs 23, 24, 25). At these sites the stems of hydroid colonies remained intact, only the hydranths (regions bearing tentacles) had been removed. Furthermore, within some crevices of the otherwise cleaned netting the basal regions of hydroids were still attached (Figs. 26, 27, 28). No difference in cleaning efficacy was observed between the soft and hard bristles.

Damage to netting

Netting cleaned with both types of bristle showed evidence of damage, with small numbers of severed filaments in many of the SEM samples. Both the soft and hard 25 mm brushes caused significant damage over the area of contact, with no apparent difference between each type. However, the soft 45 mm bristles did not cause damage.

Fouling removal and regeneration

The repeatedly cleaned area developed communities dominated by the red alga *Antithamnion* (Chapter 2, Fig. 8) and the hydroid *Syncoryne* (Fig. 9). The brush-test unit successfully removed nearly all of these types of fouling during the first three cleaning sessions (November 11, 17 and 24). On the inside and right-hand surfaces of the netting bars the majority of fouling was removed and, whilst remnants often occurred on the left-hand and upper surfaces (eg. Figs 8A, 9A, 29A, B), these caused minimal blockage of the mesh. However, this fouling regenerated quickly (Figs. 8, 9), and at 1 m the open area of the mesh decreased by 36% in 7 days, from 5.90 ± 0.07 cm^2 ($\pm$ s.e) to 3.77 ± 0.18 cm^2 ($\pm$ s.e.) (Fig. 7).

Problems with the hydraulic system on 3 December limited cleaning, and there was only a minor improvement in the mean open mesh area (Fig. 7). Some sample sites were not cleaned (eg Figs. 8D, 9D), but good cleaning was achieved at others (Figs 29A, B). The cage netting adjacent to the repeatedly cleaned area was heavily fouled and had a greater diversity of species (Figs 29C, D, E). In particular, significant amounts of tube-dwelling amphipods, the hydroid *Tubularia* and the algae *Enteromorpha prolifera* and *Porphyra lucasii* occurred only on the adjacent uncleaned sites.

Figure captions for scanning electron micrographs.

Figure 23. Netting surface after cleaning with the brush-test unit. The majority of fouling was removed from the inside surface of the netting, but significant fouling remained on the lower sides. Bar = 1 mm.

Figure 24. Netting surface after cleaning with the brush-test unit. The majority of fouling was removed from the inside surface of the netting, but significant fouling remained on the lower sides. Bar = 1 mm.

Figure 25. Hydroid colonies attached to the lower surface of a netting bar after cleaning. Bar = 1 mm.

Figure 26. Remnants of hydroids that remained in crevices after cleaning. Bar = 1 mm.

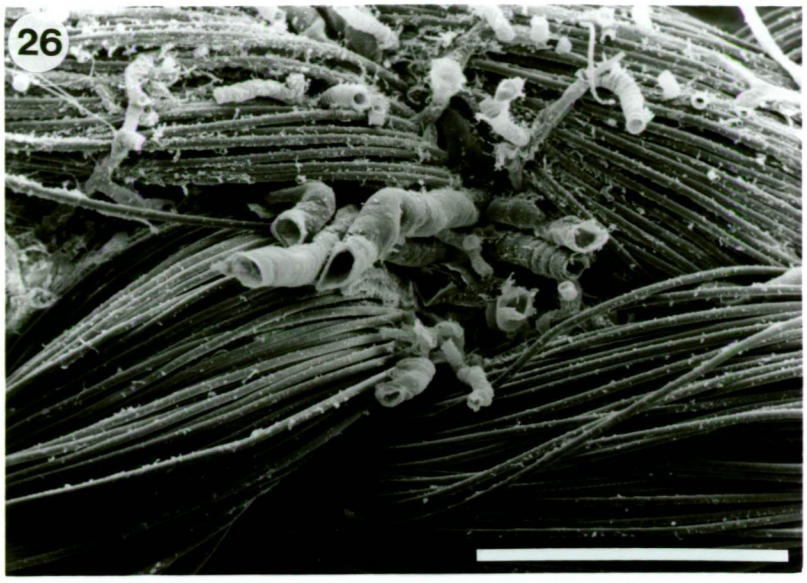
Figure 27. Remnants of hydroids that remained in crevices after cleaning. Bar = 1 mm.

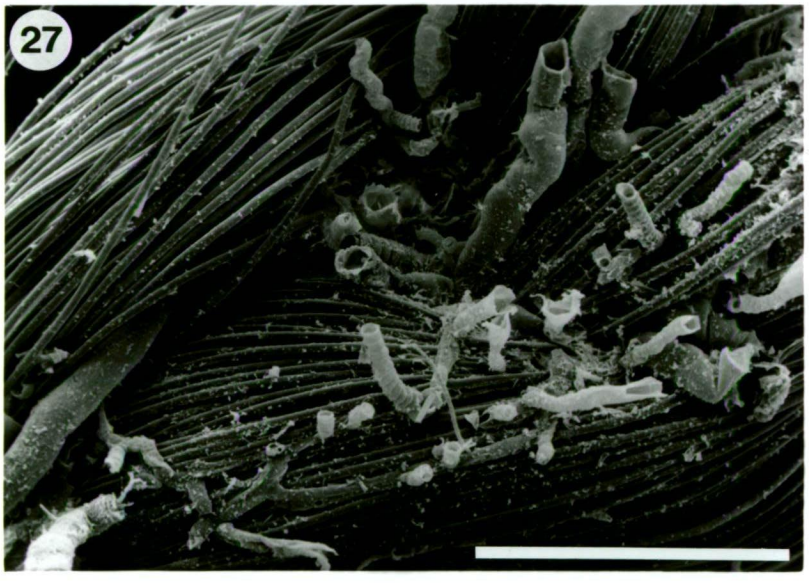
Figure 28. Basal regions of hydroids that remained attached to the netting after cleaning. Bar = 0.1 mm.


23


24


25


26


27


28

3.3.3 Discussion

Both the hydroid removal and the fouling regeneration trial demonstrated that cleaning with a rotating brush can effectively remove the majority of fouling from netting. However, remnants of fouling were observed in both trials, and small algal remnants quickly regrew and caused significant blockage of netting within 1 week (Fig. 7). The remnants of *Obelia australis* were also likely to rapidly regrow, and the regenerative capacity of hydroids is well recognised (Buchsbaum *et al.*, 1987). However, whilst those colonies that remained on the outer and lower sides of bars should recover quickly, the recovery of the small basal remnants within surface crevices (Figs 26, 27, 28) may be limited. These fast re-fouling rates demonstrate that frequent cleaning may be necessary. However, trials on other fouling communities, and during different seasons, will need to be conducted to examine the speed at which different fouling species recover, and thus determine minimum time intervals between cleaning.

The repeated cleaning of netting selected for two fouling species which regrew quickly, and reduced the diversity of fouling compared with adjacent areas of netting. Similarly, intensive non-selective grazing by fish has been found to reduce species diversity in attached algal communities by selecting for species capable of rapid recolonisation (Day, 1977). The selectivity in the cleaning trial is also likely to reflect the ability of some fouling species to quickly recover from fragmentation. Rapid algal regrowth on cleaned ship hulls has been found to occur from stimulated rhizoid growth and increased branching when *Enteromorpha* plants are severed above the basal rhizoids (Moss and Marsland, 1976a). Furthermore, small fragments of the thallus of this alga (of only a few cells) can regenerate into a new plant. A similar regenerative ability is recognised in other algae, including the green algae *Halimeda discoidae* and *Spirogyra irregularis*, the red alga *Acanthophora spicifera* and the brown alga *Acinetospora crinita* (Amsler, 1984; Kilar & Mclachlan, 1986; Walters & Smith, 1994; Chaudhary & Srivastava, 1995). In these species regeneration and rhizoid production from fragments provides a mechanism of recovery from damage due to turbulence and herbivory. Furthermore, fragmentation is an important means of propagation and distribution of these species. Amsler (1984) reported on a population of *Acinetospora crinita* which was maintained solely by vegetative fragmentation.

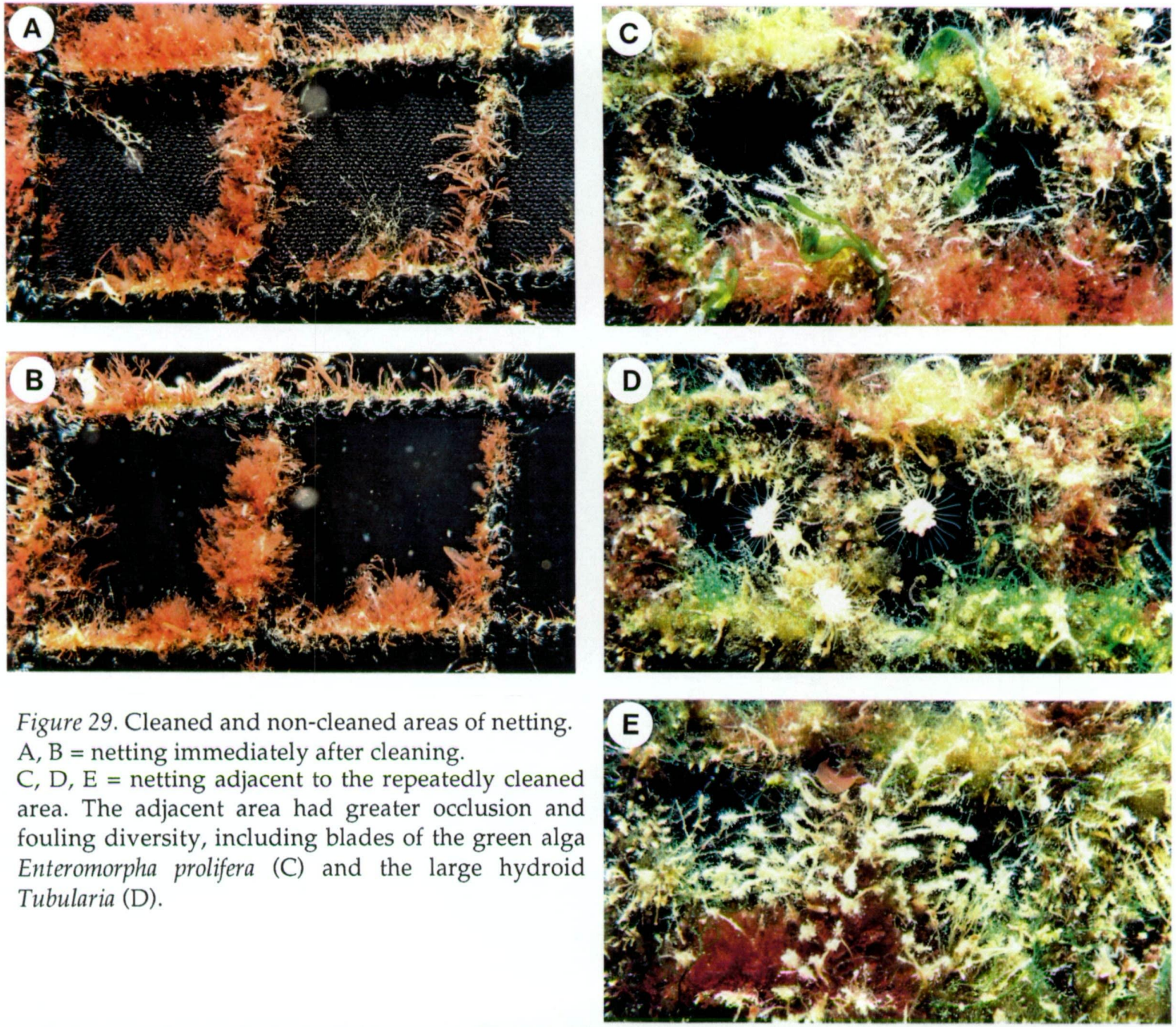


Figure 29. Cleaned and non-cleaned areas of netting.
A, B = netting immediately after cleaning.
C, D, E = netting adjacent to the repeatedly cleaned area. The adjacent area had greater occlusion and fouling diversity, including blades of the green alga *Enteromorpha prolifera* (C) and the large hydroid *Tubularia* (D).

A significant problem with operation of the brush-test unit was the need to keep the netting taut, because the bristles frequently grabbed the netting causing it to wrap around the brush. To alleviate this a diver held the net taut for the hydroid trial and the net was tied to the predator fence (which surrounded the salmon cage) for the regeneration trial. This could be solved if future units were designed with two brushes rotating in opposite directions to temporarily create a taut area of netting between the brushes. To reduce damage to the netting future cleaning units should be constructed with brushes of long, soft bristles

The use of contra-rotating brushes would also greatly reduce the quantity of remnant fouling. The location of remnant fouling showed that rotation of the brush in only one direction limits bristles contact with much of the surface. In the hydroid removal trial the brush rotated downward and remnants occurred on the lower surfaces of the netting bars (Figs 23, 24). Similarly, in the regeneration trial the unit was held vertically with the brush rotating from right to left, and the remnants occurred on the left-hand side of the bars (eg. Figs 6A, 7A, 29A, B).

Cleaning on the outside surface of a cage will not be possible on many farms because the cages are surrounded by predator netting. Consequently, whilst the majority fouling can be removed from the inside of a net, fouling growth on the outer face may eventually necessitate net replacement. However, underwater cleaning of netting will greatly extend the period of net immersion and realise significant cost savings by reducing the frequency of net changing.

3.4 Evaluation of the prototype *in situ* cleaner

3.4.1 Materials and Methods

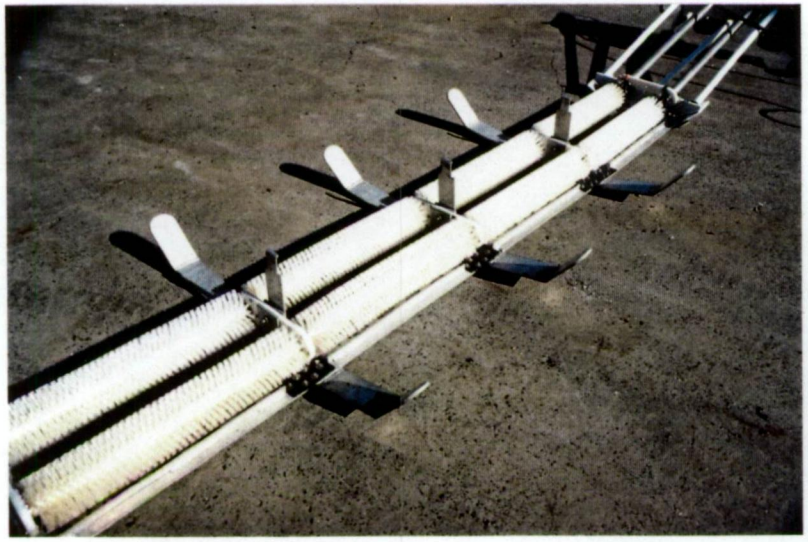
***In situ* cleaning equipment**

The cleaner was configured with 4 pairs of 800 mm long contra-rotating brushes (Fig. 30). Cages used for trials were set up so that the net sloped inward toward the cage base (5° to 10° from vertical), allowing the brushes to lie against the inside surface. Brushes were attached to a gearbox mounted on the hand rail of the cage and driven by a petrol-powered hydraulic unit. The brushes rotated at 25 revolutions/min, with the gearbox moving along the hand rail at 0.3 m/min.

Trial 1: Fouling removal and regrowth I

A 65 m circumference cage (96 ply, 22 mm bar, cleaned white netting) was immersed on October 31, 1994, and mechanically cleaned *in situ* on November 16 and December 7, 14 and 21. The western half of the cage was cleaned on November 14. On the northern and southern faces of the cage blocks were marked for sampling at 100-150 cm depth and 180-230 cm depth (Fig. 31). These blocks corresponded to the central 63% of the areas cleaned by the second and third pairs of brushes on the cleaner respectively. On the northern face one block was also marked at 20-70 cm depth to correspond with the first pair of brushes. All blocks were divided into 2 replicate sections, each 90 mesh-holes wide (2m) by 20 high. Close-up underwater photographs of the net were taken on November 24, and December 1, 6, 8, 13, 15, 20 and 22. On each of the sampling dates 12 randomly-located sites (each site 6 by 4 bars) were photographed in each section. During the last four sampling dates, all of the randomly-located sites in depths 1 and 2 on the northern face were photographed twice. Duplication of these samples was conducted to measure precision and replicability of the photographs. At depth 1 on the northern face of the cage, sampling was conducted only on the last four sampling dates. Photographs were taken using a Nikonos-V camera with two SB-103 strobes, a Nikonos close-up outfit (1:4.5) and 100ASA film. The level of mesh blockage for each sampling occasion was quantified from the photographs by image analysis (*Chapter 2*).

During all *in situ* cleaning trials no fish were stocked in the study cage. This was necessary because the netting was not to be changed during the trial and high levels of mesh occlusion (resulting in low water flow) may have arisen from ineffective cleaning and the restriction of cleaning to the upper 3.5 m. Whilst the absence of fish may have reduced nutrient levels and therefore fouling, the cage was moored close to fully-stocked cages to maximise conditions of nutrient enrichment.



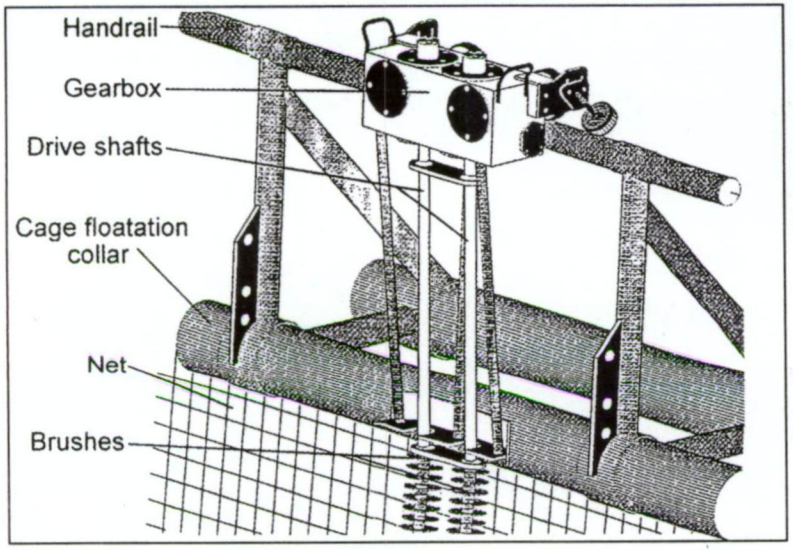


Figure 30. The prototype *in situ* net cleaner, comprised of four-pairs of contra-rotating brushes. The linkage at each pair was flexible to allow for changes in curvature of the net. The 'arms' attached at each linkage were included to prevent twisting. B = gearbox mounted on the hand rail of the cage used to rotate the brushes.

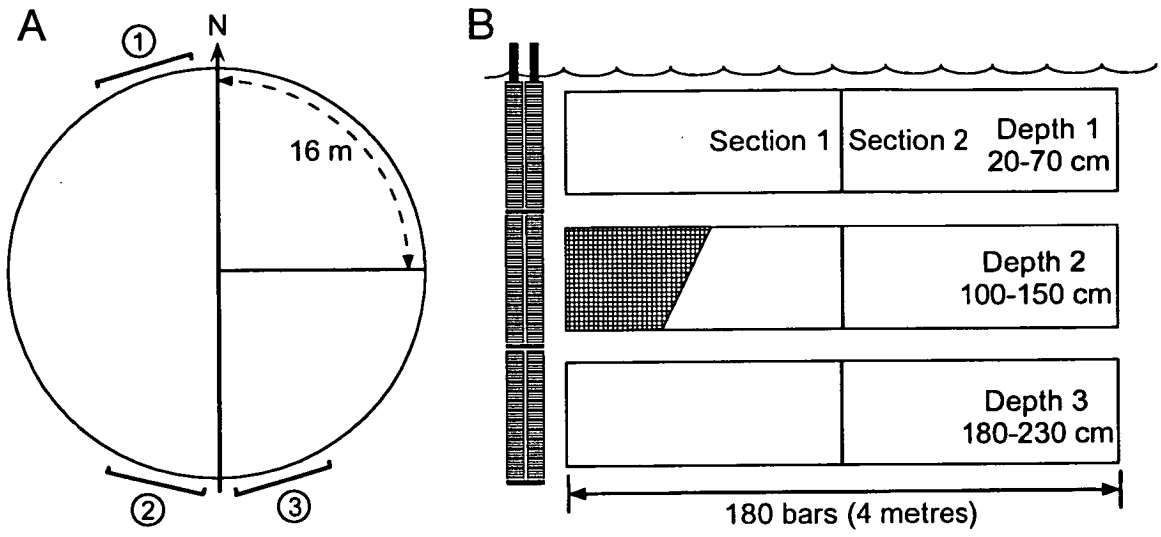


Figure 31. (A) Blocks from 100-150 cm and 180-230 cm depth were marked in each of three areas for sampling on the northern and southern faces of a 65 m cage. On the northern face a block was also marked at 20-70 cm depth. Each block was divided into 2 sections, each 90 mesh holes wide (approx. 2 m). The net was immersed on October 31 and cleaned on November 16, and December 7, 14 and 21. On December 14 area 3 was not cleaned.

Trial 2: Residual fouling, recolonisation and regrowth

The remnants of fouling after cleaning, and the re-development of fouling, were examined on regularly cleaned netting and compared with netting which had developed an established community. On November 25, 12 patches (6 by 8 bars, identical to the cage netting) were randomly attached at each of 4 depths on the southern face of the 65 m cage (Fig. 32). Within each depth the patches were placed into 2 sections, and none were adjacent. All of the patches were cleaned weekly from December 7 to January 4. In the following three cleaning sessions (January 11, 18 and 25) half the patches at each depth were cleaned. On February 3 all the patches were cleaned and three randomly selected patches were removed from every section. The remaining 24 patches were removed on February 10 to observe fouling regrowth and recolonisation after 7 days. Immediately after removal, each patch was placed on a blue sheet and photographed for image analysis. Four bars were then randomly selected from the central area of each patch, removed and prepared for scanning electron microscopy. Samples were prepared according to Hodson and Burke (1994), however, fixation with osmium tetroxide was omitted.

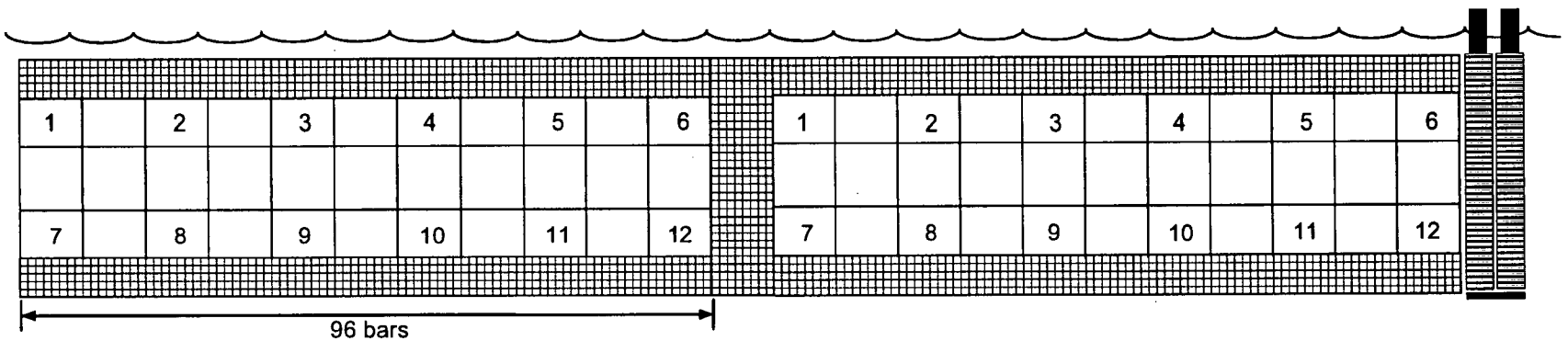


Figure 32. Placement of netting patches for observation of residual fouling, recolonisation and regowth. Two sections, each 96 bars wide, were marked for placement of patches at 4 depths (each depth corresponding to the brushes on the cleaner). Within each section 6 patches (each 6 by 8 bars) were attached, randomly located within 12 possible attachment sites.

Trial 3: Fouling removal and regrowth II

A 40 m circumference cage (120 ply, 25 mm bar, new white netting) was immersed on December 6, 1994, and mechanically cleaned *in situ* on January 18, 25 and February 1, 1995. On the northern face of the cage two large areas were marked for sampling, with each area divided into six sections (Fig. 33). One area was cleaned with one brush per depth, and the second area with two brushes per depth. Photographs of the fouling in each of the 12 sections were taken the day before and the day after each cleaning session. On each of the sampling dates 12 randomly-located sites (each 3 by 4 bars) in the sections were photographed twice. Photography and image analysis were conducted as for trial 1.

The experimental design was spatially confounded by the use of only one area per treatment. Replication within each depth was only in the form of pseudo-replicate sections. The experiment was conducted under the assumption that fouling variation between these areas was minimal. Both areas had a northerly aspect and therefore had consistent light intensity and current flow (tidal current flow was North-South). Logistic difficulties in placement of the cleaner, brush removal/addition, and the time taken for sampling precluded the use of replicate areas around the cage circumference.

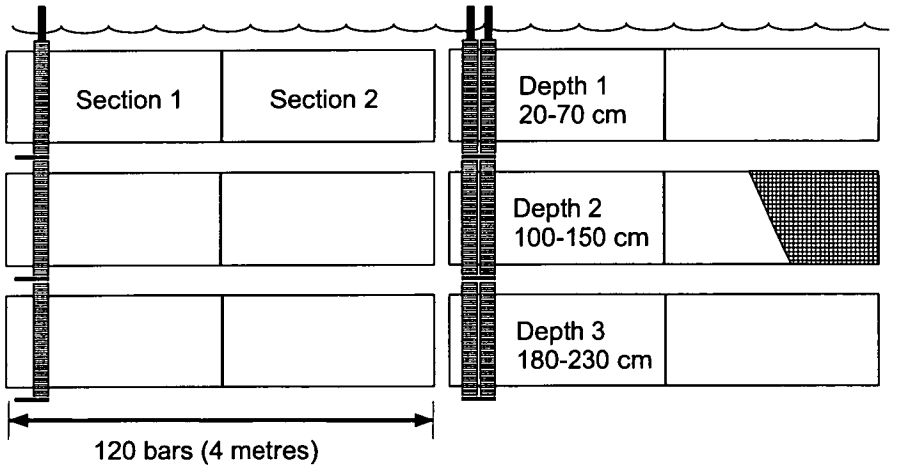


Figure 33. Two areas on the northern face of a 40 m cage were marked for sampling. The net (120 ply, 25 mm bar) was immersed on December 6, 1994, and cleaned on January 18, 25 and February 1, 1995. One area was cleaned with one brush per depth, the other with two brushes per depth.

Trial 4: Communities resistant to cleaning

Communities apparently resistant to cleaning were examined by destructive sampling of a 4 m-wide area of netting at 100-150 cm depth. Six random, non-overlapping sites (each 6 by 4 bars) were sampled before and after cleaning. All algal material at each site was removed, and the total number and length of algal blades quantified.

Trial 5: Optimum bristle length

To test the effect of brush length on cleaning efficacy, the attachment points for the brushes were modified so that the bristles could extend 5 mm further through the framework of the cleaner. Before modification, the brushes at 100-150 cm depth protruded by 20 mm and at 180-230 cm depth by 15 mm. On the northern face of a heavily fouled cage (40 m circumference, 120 ply, 25 mm bar netting), adjacent 2 m-wide areas were cleaned with brushes either in the original or modified positions. Before and after cleaning, all fouling was removed *in situ* from 12 randomly-located sites (each 3 by 4 bars) in each area from 100-150 cm and 180-230 cm depth (total number of samples = 96). The total wet weight of each species per sample area was determined by blotting the samples dry and weighing to ±0.01 g.

3.4.2 Results

Trial 1: Fouling removal and regrowth I

The development of fouling and the effects of cleaning are shown in Figs. 34-36. On the northern face (Area 1, Fig. 31) of the cage (Fig. 34) the netting rapidly became blocked after 30 days immersion. By December 13 (43 days immersion) the net was severely blocked, and only 16.6 ± 4.8 % (±s.e., n=2) of the mesh at 100-150 cm depth, and 14.3 ± 0.2 % (±s.e., n=2) of the mesh at 180-230 cm depth, remained open. In contrast, the rate and level of blockage were far less on the southern face of the cage (Fig. 35-36).

On only three occasions were there increases in the open area of mesh after cleaning in the weekly cleaned area (area 2) of the southern face (Fig. 35; A, C). In area 3 (not cleaned on December 14) a greater level of occlusion developed than in the adjacent weekly cleaned area, and cleaning resulted in an increase in open area in each section (Fig. 36). The poor efficacy of the cleaner on the southern face, and the apparent lack of cleaning at low levels of fouling on the northern face, suggested that the brushes were

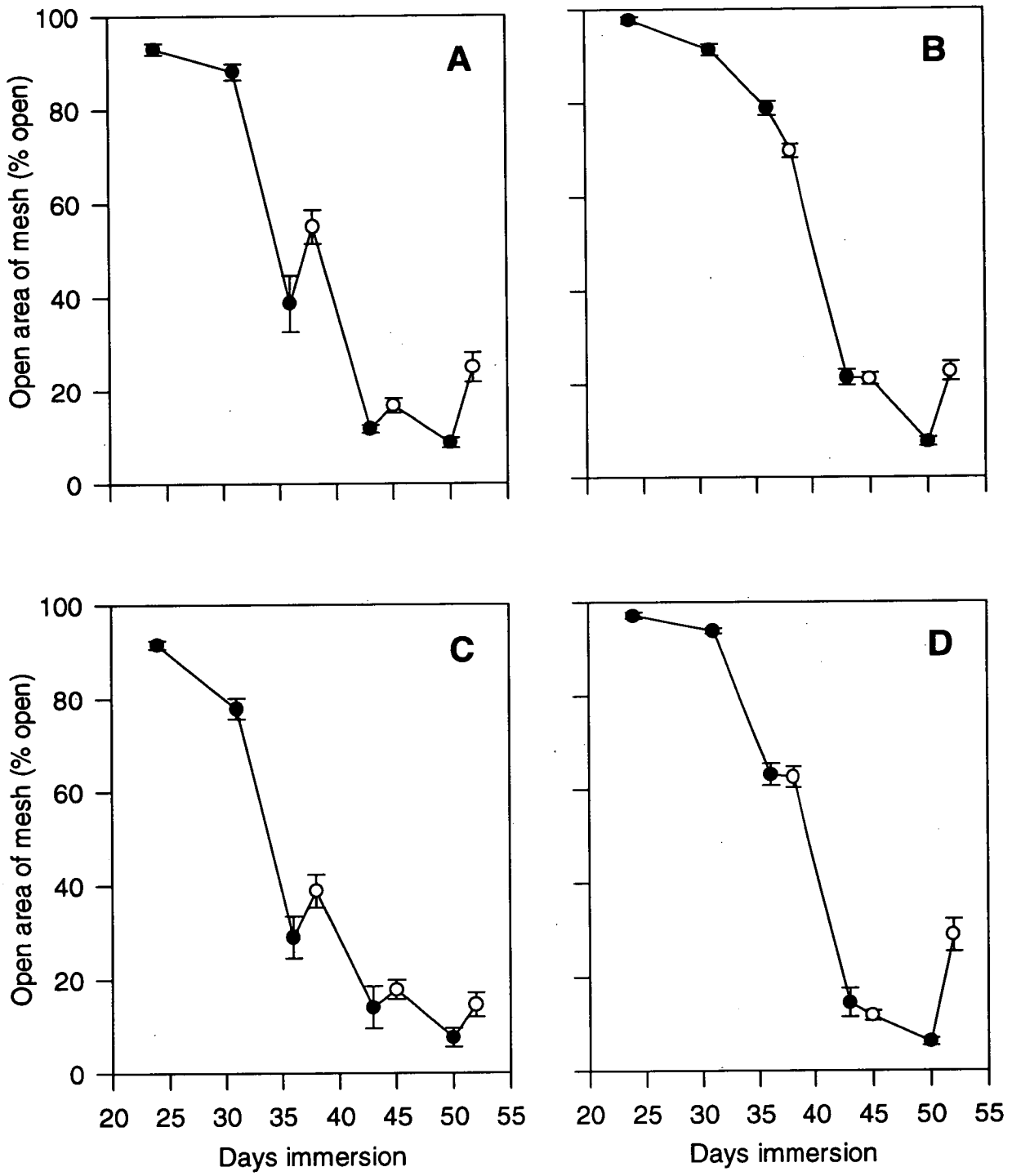


Figure 34. Levels of mesh occlusion and effect of cleaning on the northern face of the 65 m cage. A = 100-150 cm section 1. B = 100-150 cm section 2. C = 180-230 cm section 1. D = 180-230 cm section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

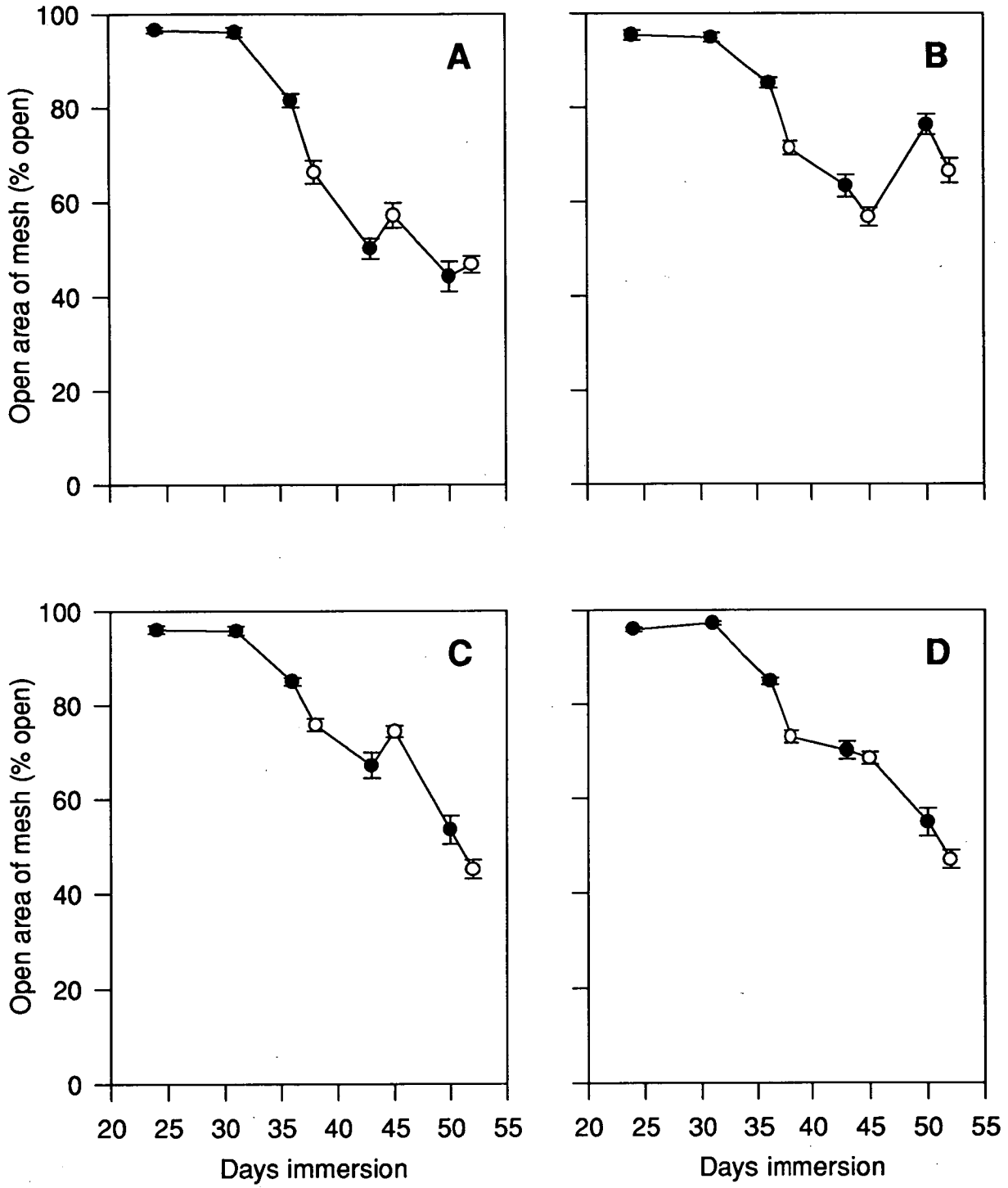


Figure 35. Levels of mesh occlusion and effect of cleaning on the southern face of the 65 m cage. A = 100-150 cm section 1. B = 100-150 cm section 2. C = 180-230 cm section 1. D = 180-230 cm section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

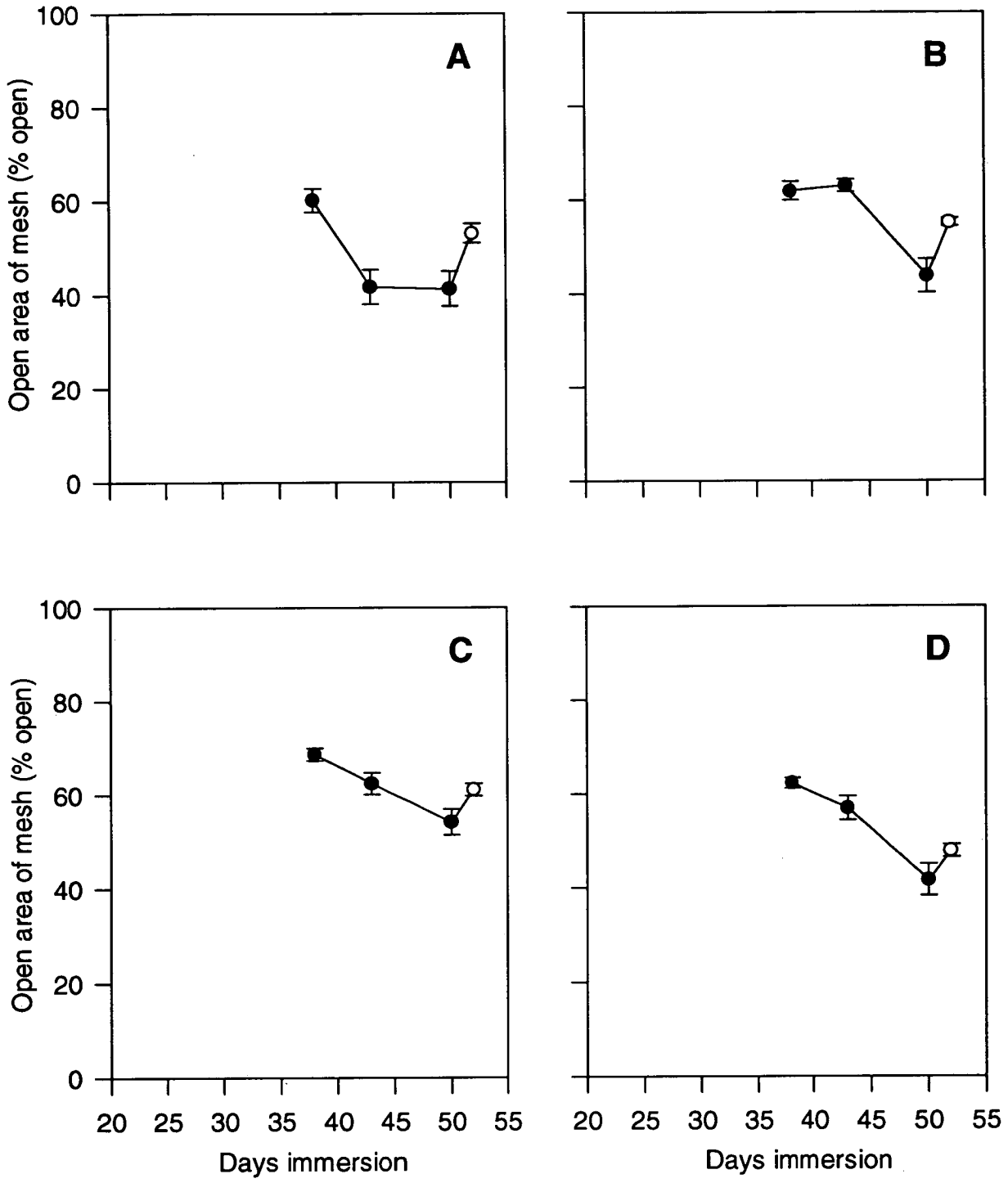


Figure 36. Levels of mesh occlusion and effect of cleaning on the southern face of the 65 m cage. This area was not cleaned on December 14.

A = 100-150 cm section 1. B = 100-150 cm section 2. C = 180-230 cm section 1.

D = 180-230 cm section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

most effective at removing fouling when fouling was greatest. This relationship was confirmed (r^2=0.59, n=28) from a plot of the percentage change in open area (from the day before to the day after cleaning) against the open area the day before cleaning (Fig. 37).

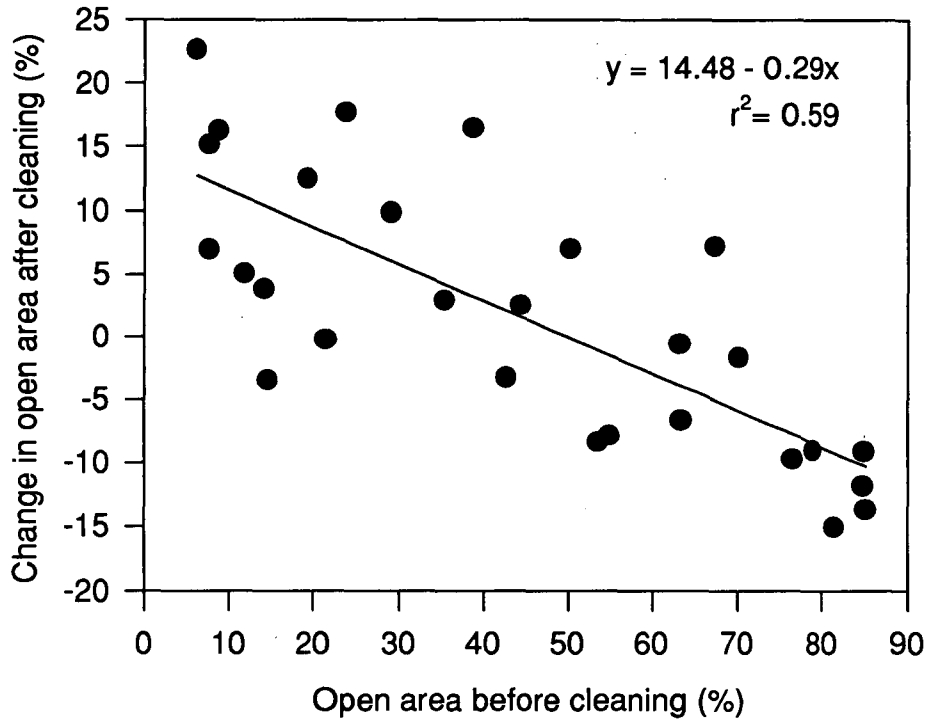


Figure 37. Relationship between the level of mesh occlusion before cleaning and the effect of the mechanical cleaner (n = 28).

Trial 2: Residual fouling, recolonisation and regrowth

Throughout this trial the net patches remained mostly free of fouling, even though the netting of the cage was heavily fouled. After cleaning (Feb 3, 70 days immersion) there was no significant difference (P=0.74) in open mesh area between those patches cleaned weekly (83.1 ± 1.9 % open, ±s.e., n=12) and those not cleaned for the previous 3 weeks (84.0 ± 1.6 % open, ±s.e., n=12). Similarly, after 7 days recovery there was no significant difference (P=0.58) between the two groups; 75.1 ± 3.9 % (±s.e., n=12) of the mesh of the weekly cleaned patches remained open, and 72.3 ± 3.0 % (±s.e., n=12) remained open in the second group.

Scanning electron microscopy also demonstrated the efficacy of cleaning of the net patches, and the lack of a significant difference between the two treatments. After cleaning, the netting surface was mostly free of both micro- and macrofouling organisms (Fig. 38). However, the brushes effectively removed organisms from the inside surface of the cage, but not from the sides of the netting bars (Fig. 38). The crevices of inside surfaces also protected fouling organisms, containing remnants of macroalgae in 50% of the samples (e.g. Fig. 39). After 7 days recovery (e.g. Figs 40, 41) the majority of samples were covered in diverse communities of diatoms, protozoa and macroalgae. These samples were often covered by debris (Fig. 40), which may have settled after the cleaning process. The level of regrowth and recolonisation on the inside surface was limited. The majority of growth which contributed to occlusion of the mesh holes developed from the sides of the bars.

Trial 3: Fouling removal and regrowth II

The open area of mesh increased in the majority of the 12 sections after each cleaning (Figs 42-44). Differences in cleaning efficacy between one and two brushes, and between the brushes mounted at each depth, were compared by a three-factor ANOVA (date, number of brushes, depth). Comparisons were made using the percent change in open area from the day before to the day after cleaning.

There was no significant difference between the number of brushes used ($P = 0.81$), with a mean increase in open area of $3.9 \pm 0.9\%$ (s.e., $n= 18$) and $4.4 \pm 1.8\%$ (s.e., $n=18$) for one and two brushes per depth, respectively. Similarly, there was no significant difference between depths ($P = 0.22$) with mean increases of $3.3 \pm 1.5\%$ (s.e., $n = 12$), $6.3 \pm 2.0\%$ (s.e., $n = 12$) and $2.9 \pm 1.6\%$ (s.e., $n = 12$) at 20-70 cm, 100-150 cm and 180-230 cm depth, respectively. However, during operation the brushes at 100-150 cm appeared to remove the greatest fouling. The inability to identify a significant difference was largely a result of the high sample variability. The comparison between depths had a power of only 0.30. The minimum detectible difference for this experimental design was 7.35% (at a power of 0.8 and $\alpha= 0.05$).

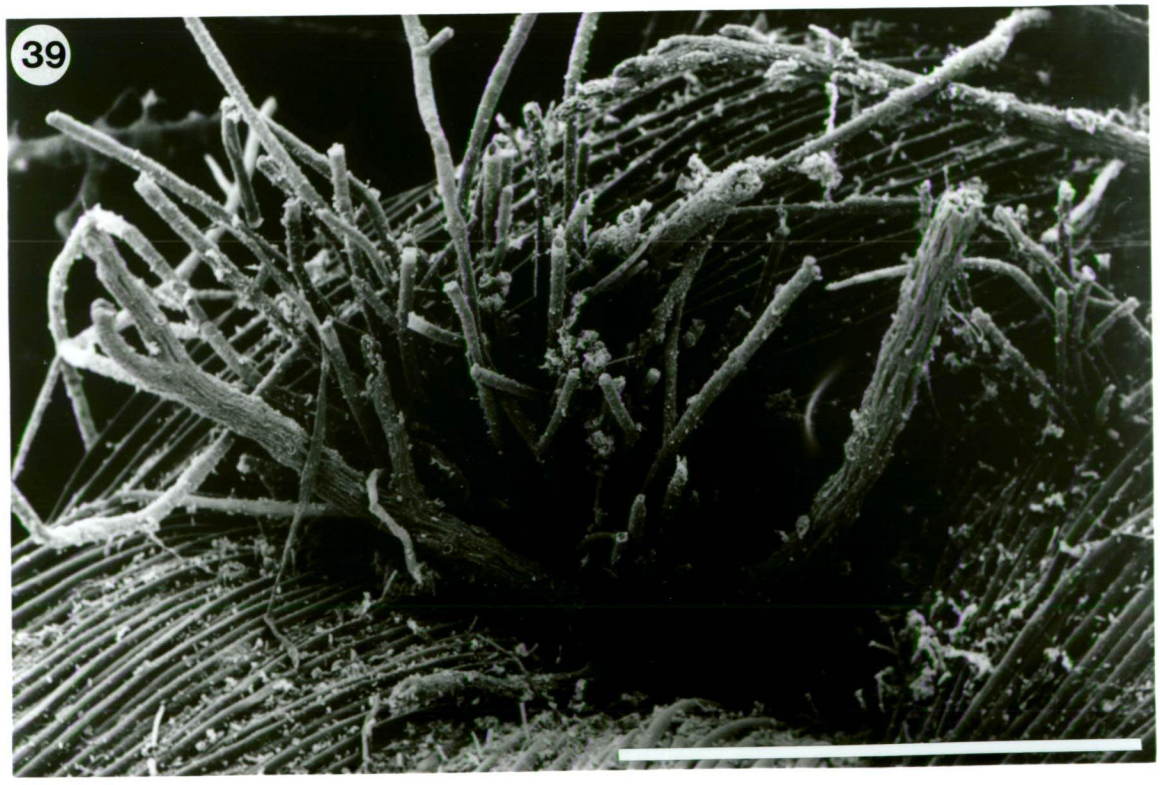
Figure captions for scanning electron micrographs.

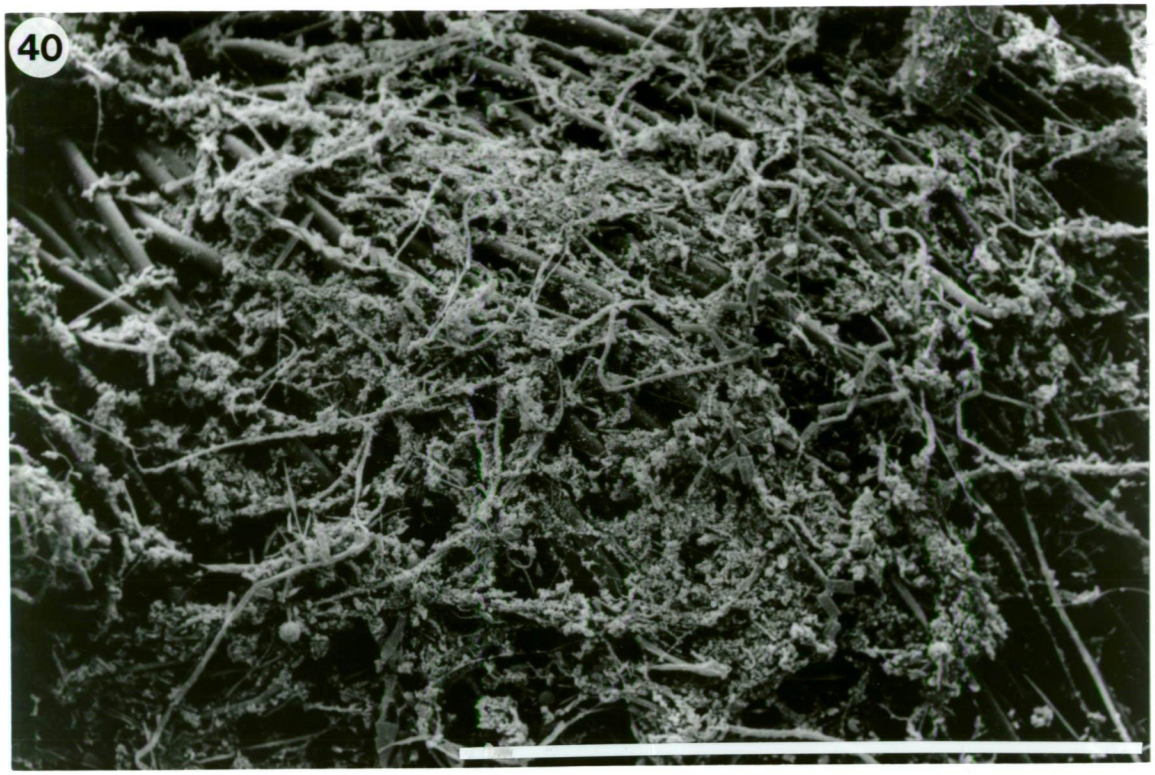
Figure 38. Netting surface after cleaning. The majority of the surface is free of macro- and microfouling, but macroalgae are still attached to the side. Bar = 1 mm.

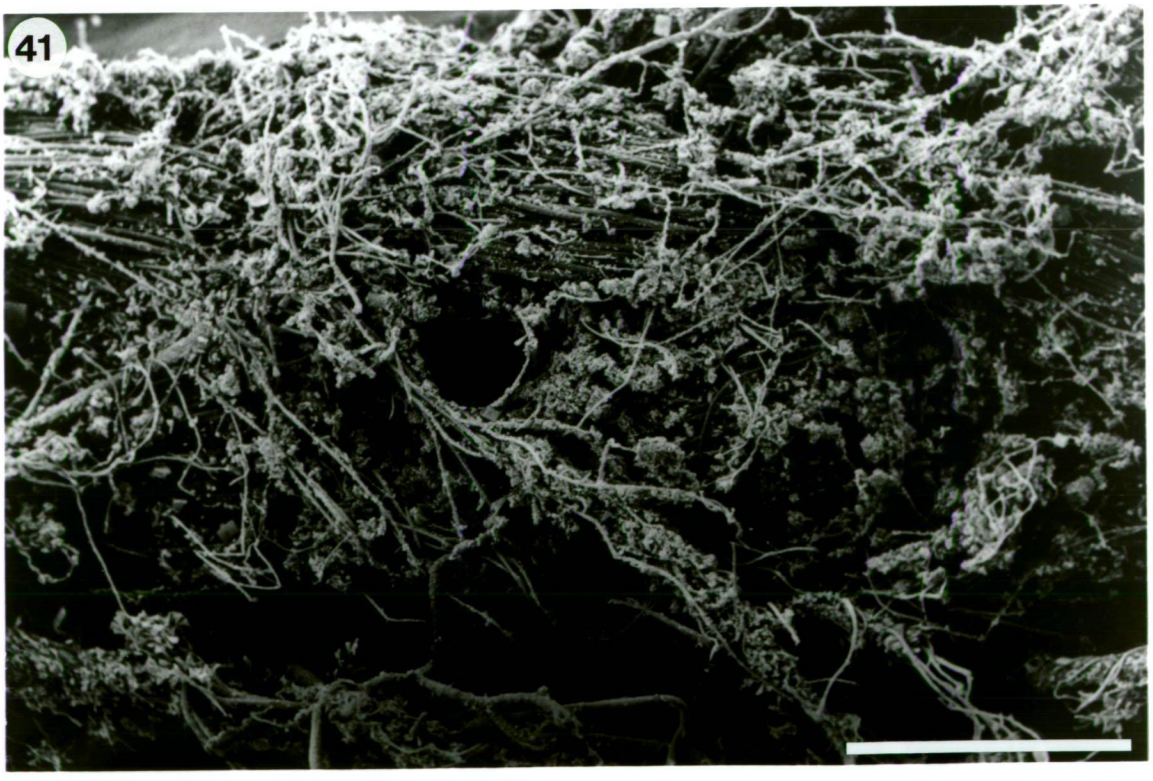
Figure 39. Remnants of macroalgae still attached to the net after cleaning. The crevices in the netting surface provided a protected region from which the fouling was not easily removed. Bar = 1 mm.

Figures 40-41. Netting surface after 7 days regrowth and recolonisation of fouling. Macroalgae, diatoms, protozoa and an accumulation of particulate matter covered the majority of samples. Bars = 1 mm.


38


39


40


41

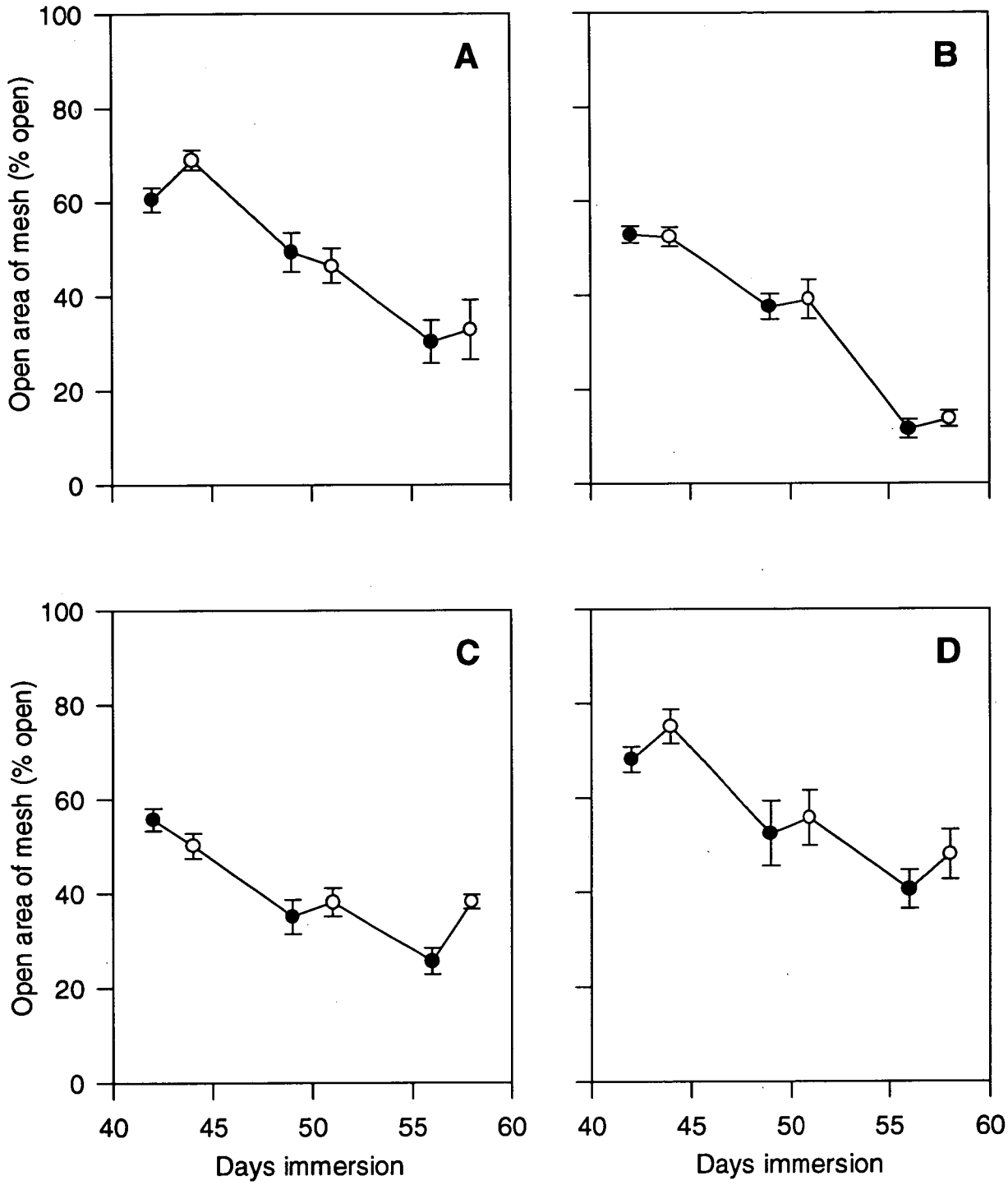


Figure 42. Levels of mesh occlusion and effect of cleaning at 20-70 cm depth. A and B = cleaned with 1 brush. C and D = cleaned with 2 brushes. A = section 1. B = section 2. C = section 1. D = section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

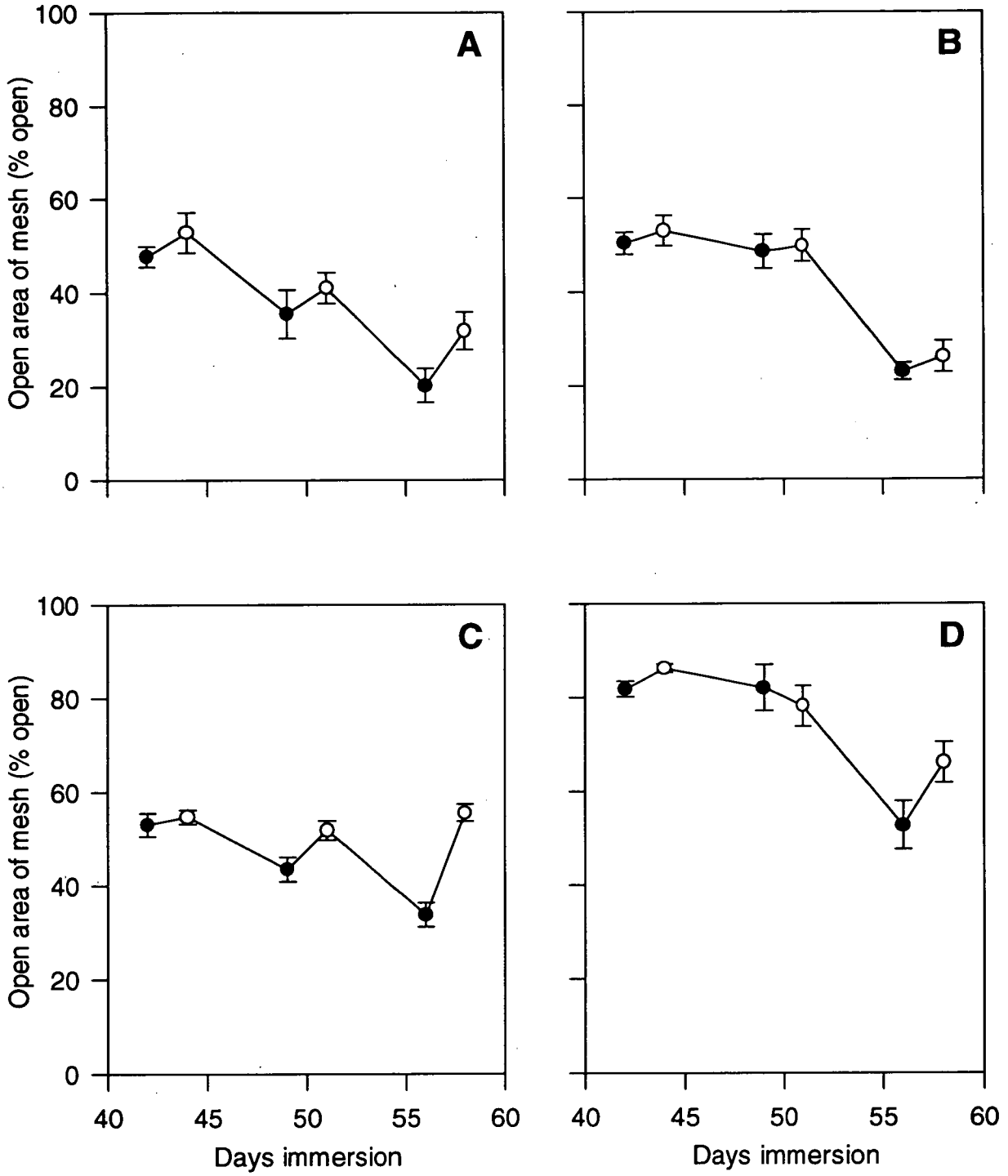


Figure 43. Levels of mesh occlusion and effect of cleaning at 100-150 cm depth.
A and B = cleaned with 1 brush. C and D = cleaned with 2 brushes. A = section 1. B = section 2.
C = section 1. D = section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

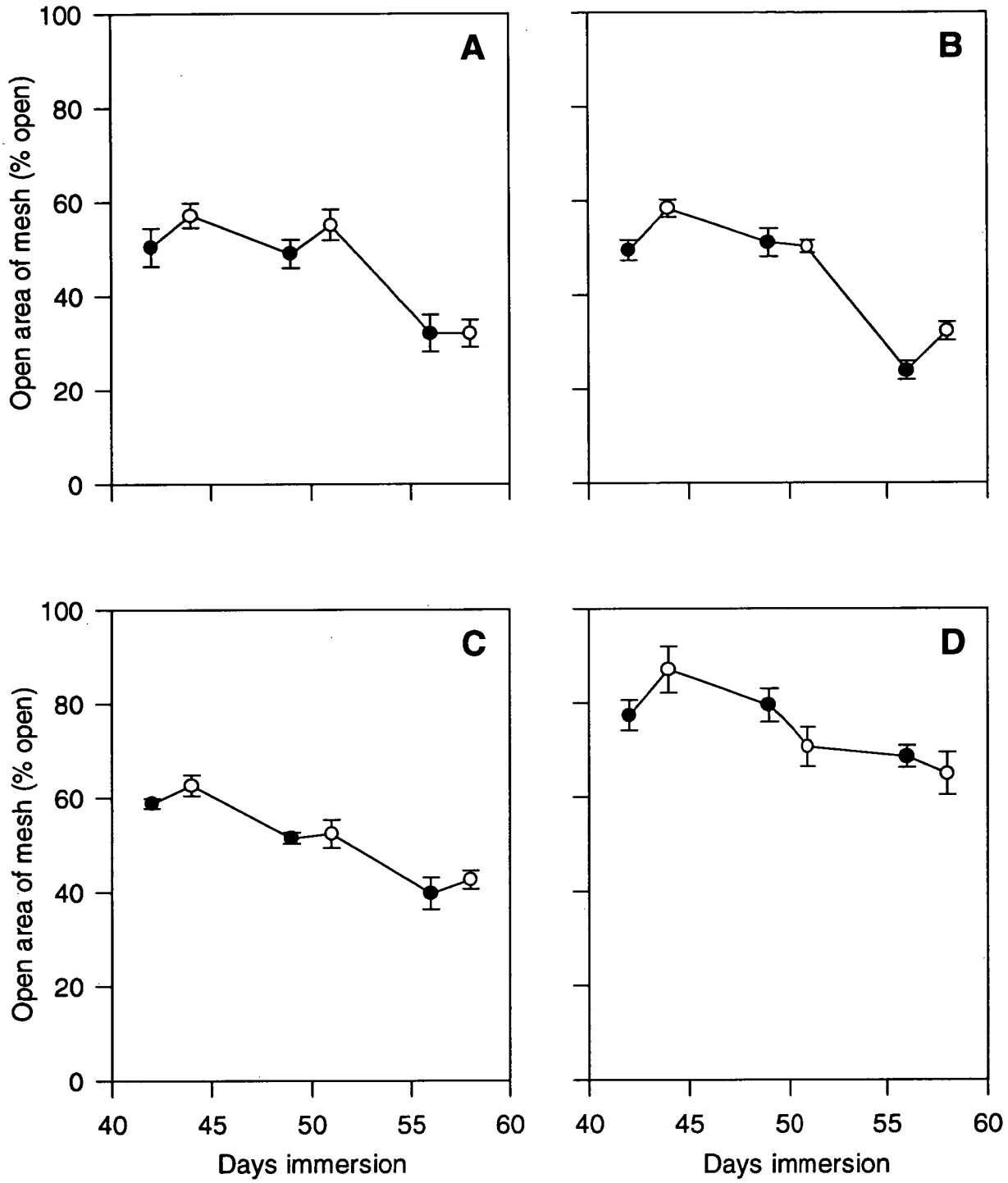


Figure 44. Levels of mesh occlusion and effect of cleaning at 180-230 cm depth.
A and B = cleaned with 1 brush. C and D = cleaned with 2 brushes. A = section 1. B = section 2.
C = section 1. D = section 2. Open symbols = day after cleaning. Bars = standard error, n = 12.

A significant relationship (P = 0.03) was found between the percent increase in open area and the level of occlusion before cleaning (Fig. 45). That is, as for Trial 1, the cleaner produced the greatest improvement in mesh area at the higher levels of fouling.

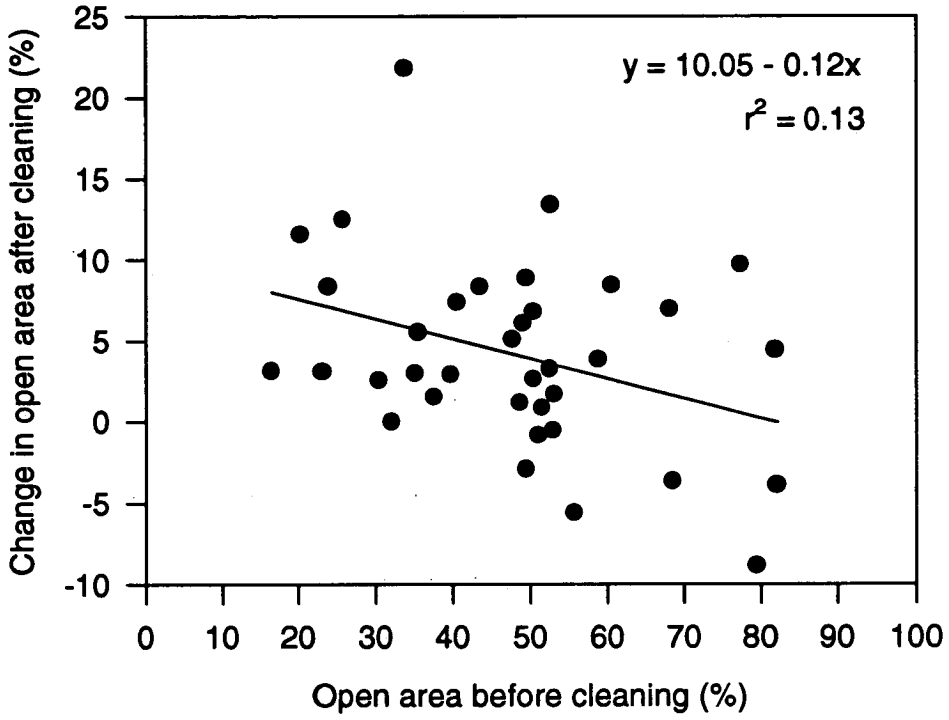


Figure 45. Relationship between the level of mesh occlusion before cleaning and the effect of the mechanical cleaner (n = 36).

Trial 4: Communities resistant to cleaning

By January 12 a dense community of the brown alga *Scytosiphon lomentaria* had developed on the 65 m cage (73 days immersion) (Fig. 46). The *in situ* cleaner had little impact on this fouling community. There was no significant difference in numbers of plants per sample area (112 ± 5 before, 106 ± 4 after; ±s.e., P=0.45, n=6), or in the average length of the plants (4.6 ± 0.3 cm before, 5.2 ± 0.3 cm after; ±s.e., P=0.14, n=6). *S. lomentaria* grew as long, thin blades, that were securely fixed to the net. During cleaning these blades were seen to lie flat on the netting and so were not grabbed and removed by the brushes.

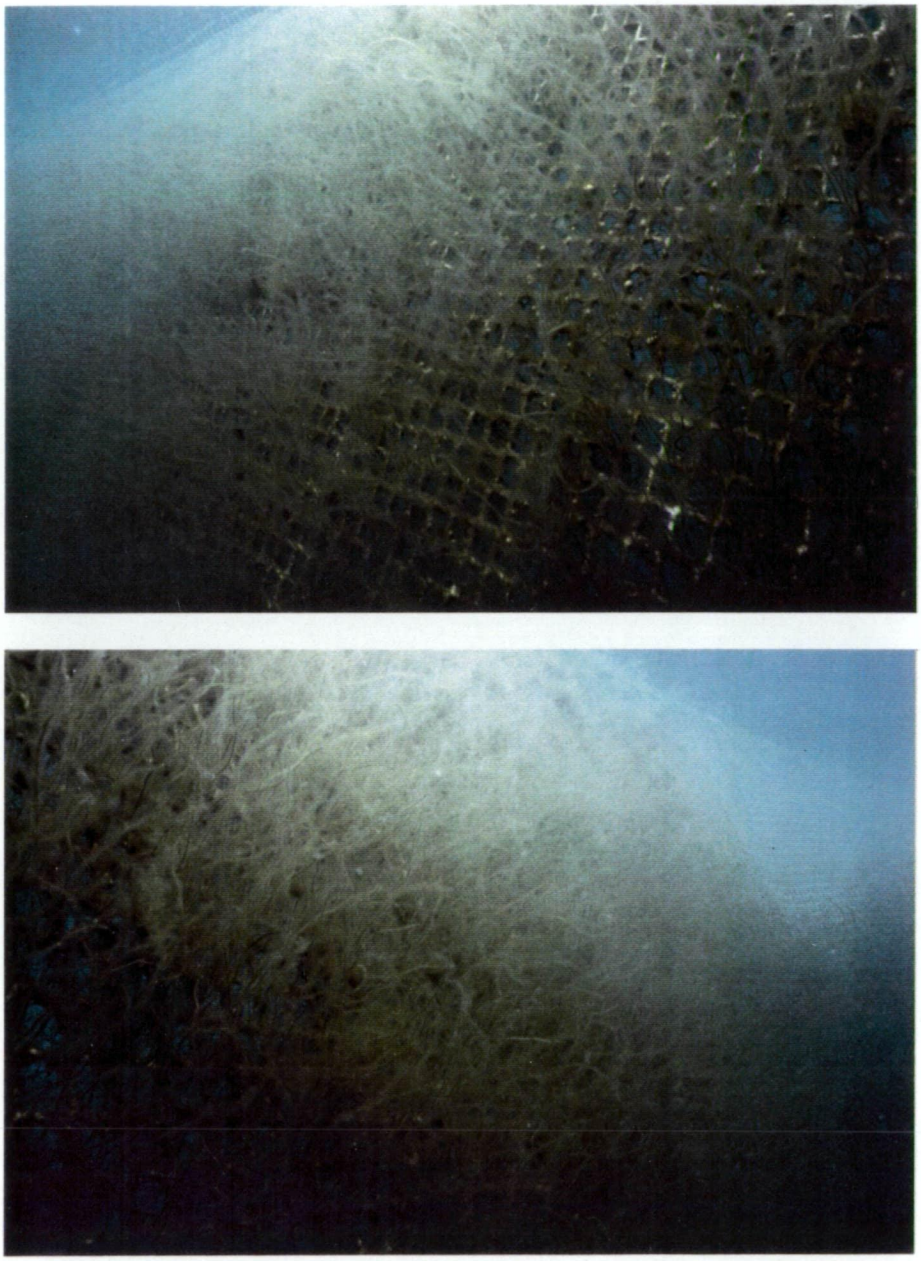


Figure 46. Dense community of *Scytosiphon lomentaria* fouling the repeatedly cleaned cage. The blades of this alga lay flat on the netting during cleaning and were not grabbed by the brushes.

Trial 5: Optimum bristle length

A significant reduction in mean fouling biomass was observed in each sample area after cleaning, irrespective of bristle length (Fig. 47). At 100-150 cm depth the modification of brush positions did not significantly change the biomass remaining after cleaning (1.23 ± 0.22 g original, 1.87 ± 0.25 g modified). However, at 180-230 cm depth, the modification of brush positions did significantly improve cleaning efficacy (3.32 ± 3.2 g original, 0.80 ± 0.23 g modified).

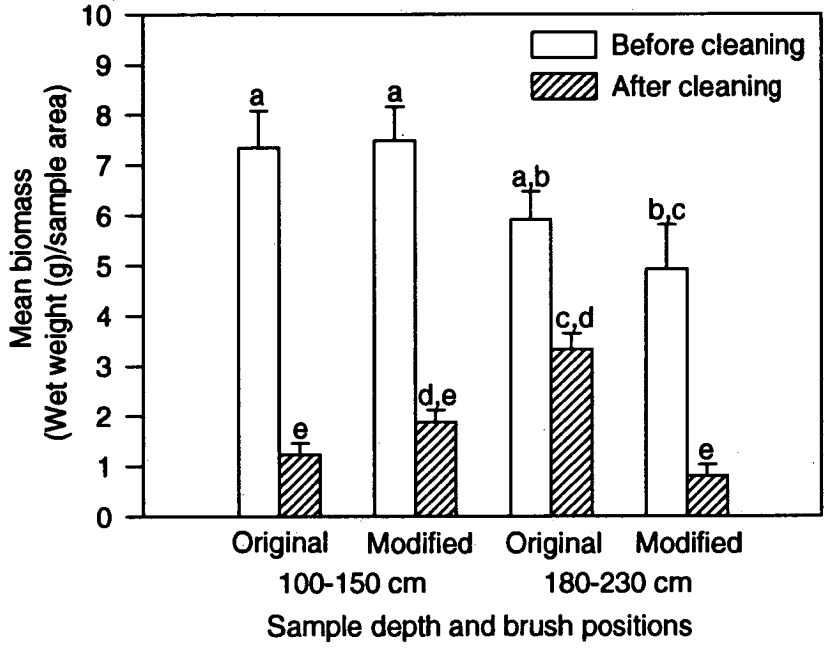


Figure 47. Reduction in fouling biomass after cleaning with brushes in the original and modified positions. Each column represents the mean of 12 samples. Bars = standard error. Columns sharing the same letter are not significantly different (P > 0.05, Ryan's test).

There was no significant difference in mean biomass (before cleaning) between the two sample areas at 100-150 cm depth, or in the two sample areas at 180-230 cm depth. Consequently, the data within each depth were combined to identify differences in community structure between depths (Table 6). Similarly, there was no significant difference in mean biomass in the two areas after cleaning at 100-150 cm depth, and all data was combined to identify the effects of cleaning on each species (Table 6). The fouling community was comprised of two Bryozoa, *Scrupocellaria bertholettii* and

Bugula neritina, two species of brown algae, *Asperococcus bullosus* and *Cutleria multifida*, and four species of red algae, *Ceramium tasmanicum*, *Polysiphonia infestans*, *Brongniartella australis*, and *Chondria fusifolia*. At both depths the only species to occur in all sample areas was *C. fusifolia*. Biomass comparisons for this species, between each depth and before and after cleaning, were made using a t-test. However, the same comparisons for all other species were conducted with a Mann-Whitney U test because of the lack of normal distribution.

A significantly greater biomass of the alga *C. fusifolia* and the bryozoan *S. bertholettii* occurred at the shallower depth. At both depths *C. fusifolia* accounted for the majority of biomass. At 100-150 cm depth the effects of cleaning differed between species (Table 6). *A. bullosus*, *C. tasmanicum* and *C. multifida* were not found in any sample area after cleaning, and both *C. fusifolia* and *P. infestans* were significantly reduced after cleaning. However, no significant reduction in biomass of the alga *B. australis* or either of the bryozoans occurred.

Table 6
Fouling biomass, occurrence and reduction after cleaning (24 sample areas per depth)

	100-150 cm depth		After cleaning, 100-150 cm depth		180-230 cm depth	
Taxa	Biomass g/sample area (mean ± s.e.)	Occurrence, % of samples with species	Biomass ratio before: after	Occurrence, % of samples with species	Biomass g/sample area (mean ± s.e.)	Occurrence, % of samples with species
Algae						
Asperococcus bullosus	0.02 ± 0.02	8	1: 0	0	0.03 ± 0.03	4
Brongniartella australis	0.04 ± 0.01	46	1: 0.59	21	0.07 ± 0.03	54
Ceramium tasmanicum	0.01 ± 0.01	17	1: 0	0	0.06 ± 0.03	29
Chondria fusifolia	6.39 ± 0.50	100	1: 0.21[a]	100	4.84 ± 0.52[b]	100
Cutleria multifida	0.48 ± 0.26	38	1: 0	0	0.23 ± 0.15	25
Polysiphonia infestans	0.33 ± 0.11	75	1: 0.08[a]	54	0.14 ± 0.05	71
Bryozoa						
Bugula neritina	0.07 ± 0.03	42	1: 0.36	29	0.02 ± 0.01	21
Scrupocellaria bertholettii	0.07 ± 0.02	54	1: 2.09	54	0.02 ± 0.01[b]	21
Total biomass	7.41 ± 0.49				5.41 ± 0.53[b]	

[a] significant difference in biomass after cleaning ($P < 0.05$)
[b] significantly different from 100-150 cm depth ($P < 0.05$)

3.4.3 Discussion

The present design for an *in situ* cleaner was chosen because of its potential to be light weight and energy efficient. Placement of the cleaner on the hand rail of a polar-circle type fish pen provided a track on which the system could move and supported the non-submersible power supply. The configuration of the brushes allowed for the easy addition of more brushes, and therefore a greater depth of cleaning, and permitted the system to flex with the curvature of a net. The cleaner was not intended to be a complete solution to biofouling, and is unable to operate on the outside or the bottom areas of a cage. Rather, it was designed to allow extended periods of net immersion.

The initial trial of the cleaner showed that it was ineffective at reducing the blockage of netting (e.g. Fig. 34). Any reduction of blockage only occurred at high levels of fouling (Fig. 37), because the brushes had limited contact with the net as it was displaced away from the cleaning head during operation (Fig. 48). Under these conditions the cleaner successfully removed or reduced the algal fouling in trial 5 (Fig. 47), but did not significantly affect bryozoa, which were much more compact (Table 6). Light scrubbing also encouraged the development of a resistant community (Trial 4), which could lay flat against the netting during cleaning and thus was not significantly removed.

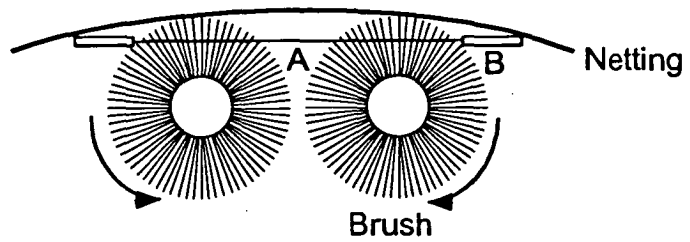


Figure 48. Displacement of netting away from the brushes limited the contact between bristles and netting. Steel cables laced between brushes (A) and bars running down the side of the brushes (B) prevented netting from wrapping around the outside of the brushes.

Modification of the cleaner to increase brush protrusion through the steel lacing by 5 mm (Fig. 48, A), indicated that 20 mm is the optimum length for this net configuration (Fig. 47). However, trial 2 demonstrated that, if the netting was not displaced from the brushes, then the cleaner could remove the majority of fouling and maintain a low level of mesh blockage. Patches of netting attached to the cage were cleaned more effectively than the net itself, because they were raised off the cage surface and subjected to harsher scrubbing.

Remnants of macroalgae on the sides of netting bars (Fig. 38) and in the crevices of the net weave (Fig. 39), showed the difficulties of removing fouling from this type of structure. Removal of fouling from the sides of netting bars is limited by how far bristles can penetrate through the mesh. Removal of small structures from crevices is also limited by bristle penetration, but might be improved by harsher scrubbing or different types of bristles. However, any approach to mechanical cleaning must consider material strength. Procedures for cleaning ship hulls, which are in part aimed at rejuvenating antifouling systems, must ensure there is no damage to the surface coating (Fischer *et al.*, 1984). Similarly, with mariculture cages there must be a compromise between the severity of scrubbing, and prevention of damage to netting. Preliminary trials with the brush-test unit showed that very stiff bristles were effective at fouling removal, but caused significant damage to nets, whilst softer bristles (used in the prototype cleaner) effectively removed fouling without damaging the net.

The large amount of debris created during cleaning, and the survival of macroalgal remnants in crevices, enabled rapid recolonisation and regrowth of fouling. Moss and Marsland (1976a) found that cleaning caused fragmentation of algal reproductive cells which released large numbers of swarmers that could immediately recolonise a surface. Recolonisation is likely to be exacerbated on cages because the structure of multifilament netting (Fig. 38) may entrap suspended material and spores of *Ectocarpus* and *Enteromorpha* (both common algae on salmon cages) preferentially settle in crevices (Crisp, 1984). Remnants of macroalgae, both attached (eg. Fig. 39) and suspended, may also rapidly regenerate. For example, Moss and Marsland (1976b) observed enhanced growth and increased thallus branching in *Enteromorpha* plants severed above the basal rhizoids. They also found that severed fragments rapidly regrew into new plants. Hodson (1994) found remnants of the red alga *Antithamnion* and the hydroid *Syncoryne* rapidly regrew on repeatedly cleaned netting. The problems of recolonisation and rapid regrowth may necessitate frequent *in situ* cleaning and furthermore, changes in species composition are likely to occur (eg. development of communities resistant to cleaning).

The development and operation of *in situ* cleaning equipment will require both debris removal and changes in cage design. Even under light levels of fouling the cleaner produced large volumes of fine particulate matter. In addition to the problem of recolonisation, the debris may irritate fish gills or disperse potential pathogens, and may lead to increased loading of nutrients and organic matter in the immediate environment. Under fouling levels of 0.5 kg/m^2 wet weight (e.g. Table 6) an 80 m cage (2000 m^2

surface area) would support 1 tonne of fouling. To recover debris a housing would need to be mounted around the brushes from which water and debris could be pumped to the surface. At the surface a filtration and debris storage system would be required, probably towed behind the cleaner on a floating platform. However, the level of fouling under which the cleaner could operate may become limited by the maximum water flow through the filter, especially a filter suitabe for recovery of fine particulate matter.

The cages used for testing the *in situ* cleaner were modified for the purpose. All folds in the nets were laced, and weights were attached to the base of the cage to create a taut surface and to ensure the netting sloped inward. Any extraneous structures on the hand rail of the cages (e.g. bird netting, mooring lines, poles for automatic feeding systems) were removed to allow unobstructed passage of the cleaner. The development of an effective system for fouling removal will therefore require a process of evolution in both net cleaner and cage design. As part of this process, "foul-release" silicone coatings that may improve cleaning efficacy and reduce power requirements are evaluated in Chapter 4.

The results of the present study show that, with good contact between brushes and netting, the *in situ* cleaning of netting is highly effective. However, the efficacy of the present cleaner was limited by its design and by the flexibility of netting, which reduced the severity of scrubbing. Under these conditions, a resistant algal community developed which could lay flat against the netting. This effectiveness was further compromised because residual structures of fouling organisms and the large quantity of debris led to rapid regrowth and recolonisation.

CHAPTER 4

Mariculture antifouling:

Silicone "foul-release" coatings

4.1 Introduction

The prevention of fouling on mariculture structures is complicated by the choice of net material and the dangers of toxins to cultured species. Multi-filament netting material is an ideal substrate for fouling; it is non-toxic, contains many crevices which can entrap and protect settling organisms, and has a high surface-area to volume ratio. Whilst copper-based antifoulants have proved effective on net cages, their use is undesirable because of environmental effects from broad-spectrum metal-based toxins, and real or perceived consumer concerns that may jeopardise market image (Lewis, 1994b). Adverse effects of metal-based antifouling have been shown for many non-target species (Lewis, 1988), and high levels of tributyltin (TBT) have been found in the muscle of salmon grown in TBT-antifouled netting (Short & Thrower, 1986; Davies & McKie, 1987) and in many fish species exposed to ship antifouling (Kannan *et al.*, 1995a, b, c). Lewis (1994b) recommended six criteria for antifouling strategies in the aquaculture industry. They should: (1) be effective against a broad range of fouling taxa, (2) be environmentally benign, (3) have no negative effects on the cultured species, (4) leave no residues in the cultured species, (5) be able to withstand on-shore handling and cleaning, (6) be economically viable.

Internationally, the development of environmentally acceptable antifoulants is focussed on two strategies: the production of "foul-release" surface coatings and coatings that release non-toxic compounds which act as deterrents (Clare *et al.*, 1992). The former strategy is based on a physical surface effect, where low surface-free-energy (low surface tension) prevents adhesion or reduces adhesion strength of fouling organisms (Mussalli, 1989; Lewis, 1994a). Substantial fouling is prevented because large masses slough from the surface and are easily removed by light cleaning or current velocities > 9 m/s (Mussalli, 1989; Baier & Meyer, 1992). The most successful coatings are presently based on silicone elastomers, and often include oil-based additives that further improve fouling resistance (Lewis, 1994a; Nevell *et al.*, 1996; Swain & Schultz, 1996). Coatings based on other materials including fluoropolyurethanes (Bultman & Griffith, 1994), polyurethanes (Wynne *et al.*, 1993) and perfluorinated polymers (Lindner, 1994) have also displayed excellent adhesion resistance.

The following chapter describes the evaluation of a commercial silicone coating (Viridian 2000, International Paints) as applied to fish-cage netting. The development, composition and adhesion of fouling is compared between the white silicone-coated netting, white uncoated netting and black uncoated netting. The preferential settlement of fouling species in relation to substrate colour, and the adaptation of species to low-surface-free energy, is described and discussed. The flexibility and non-toxic properties of silicone coatings make them highly suitable for fish-cage netting and the results show that they would greatly increase the success of *in situ* cleaning. They may also reduce fouling on predator netting because problematic species such as solitary ascidians were unable to remain attached.

4.2 Description of the field site

Trials were conducted at the Huon Aquaculture Company's lease at Hideaway Bay (Fig. 49), which is situated approximately 8 km north of the study site for previous trials (Fig. 20). The site is fully marine, except for a 2 - 5‰ salinity drop to 1 m depth after high rainfall in winter. Water temperatures range from 11°C to 17°C (Fig. 50). Water movement is dominated by tidal flow and current speed varies from 5 to 20 cm/s (Anon, 1996e).

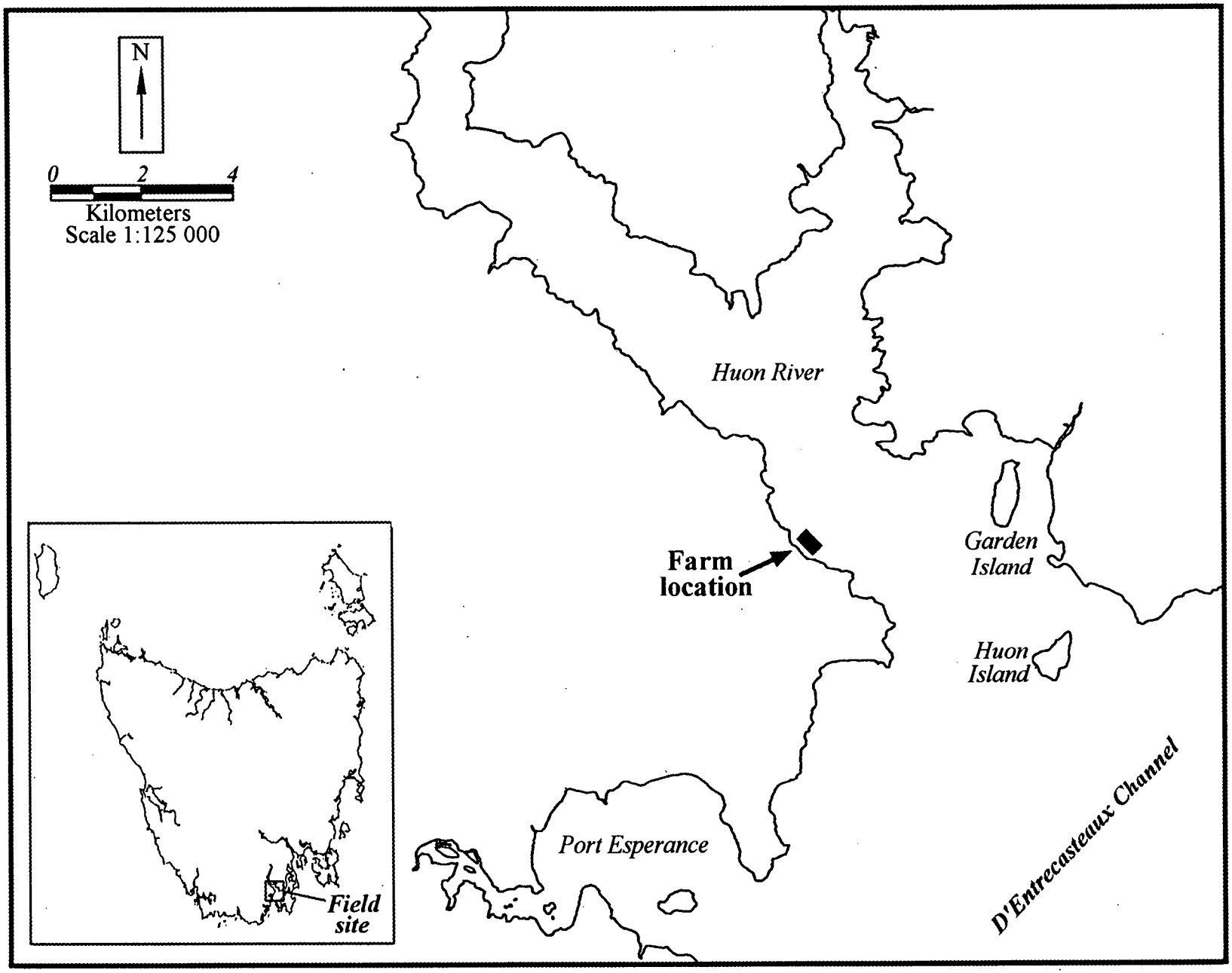


Figure 49. Location of field site for evaluation of the silicone coating Viridian 2000.

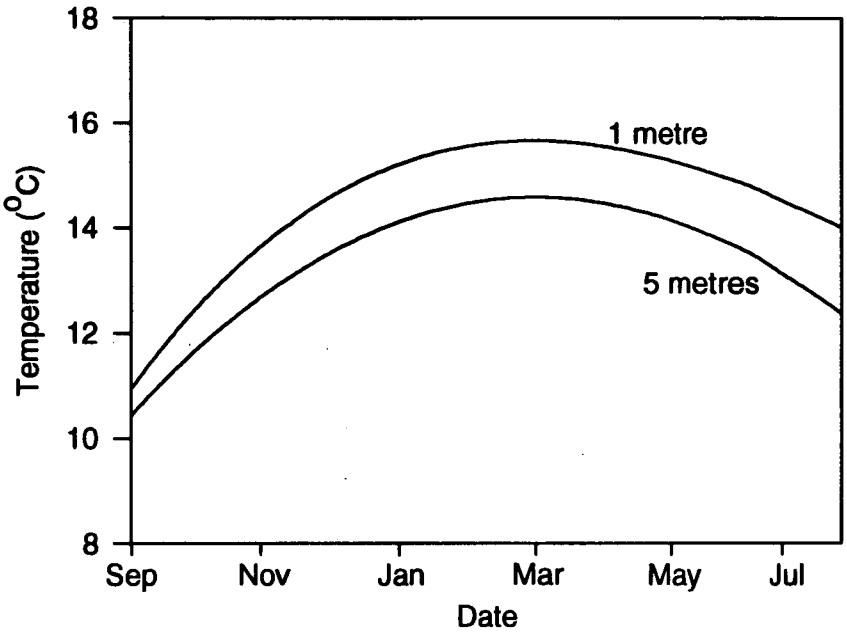


Figure 50. Mean daily temperature at 1 and 5 metres depth at the Huon Aquaculture Company lease (Hideaway Bay, Tasmania).

4.3 Materials and Methods

4.3.1 Panel and raft design

On September 8, 1995, 21 panels (each 1 m X 1m, 96 ply, 20 mm bar netting) were immersed to evaluate the silicone-based product Viridian 2000 (International Paints). Seven replicates were used for each of 3 netting types: (1) silicone-coated netting, (2) uncoated white netting and (3) uncoated black netting. Viridian 2000 consists of two coats with a white pigmented tie-coat and a clear finish. Nets were coated by repeated immersion in a bucket and the tie coat was dried for 3 hours prior to application of the finish. The finish was dried for 48 hours before attachment of netting to panels. A volume of 3.8 L (1 US Gallon) was sufficient to coat 9 m^2 of net. A white tie-coat was used and applied to black netting to aid observation of cracking and coating loss.

The panels were attached to a 9.5 m X 6 m raft tethered within a 65-metre-circumference unstocked salmon cage (Fig. 51). Panels were placed in a 1-way block design with each of the three treatments placed randomly within the seven support beams of the raft. This design was chosen to compensate for the north-south current flow and

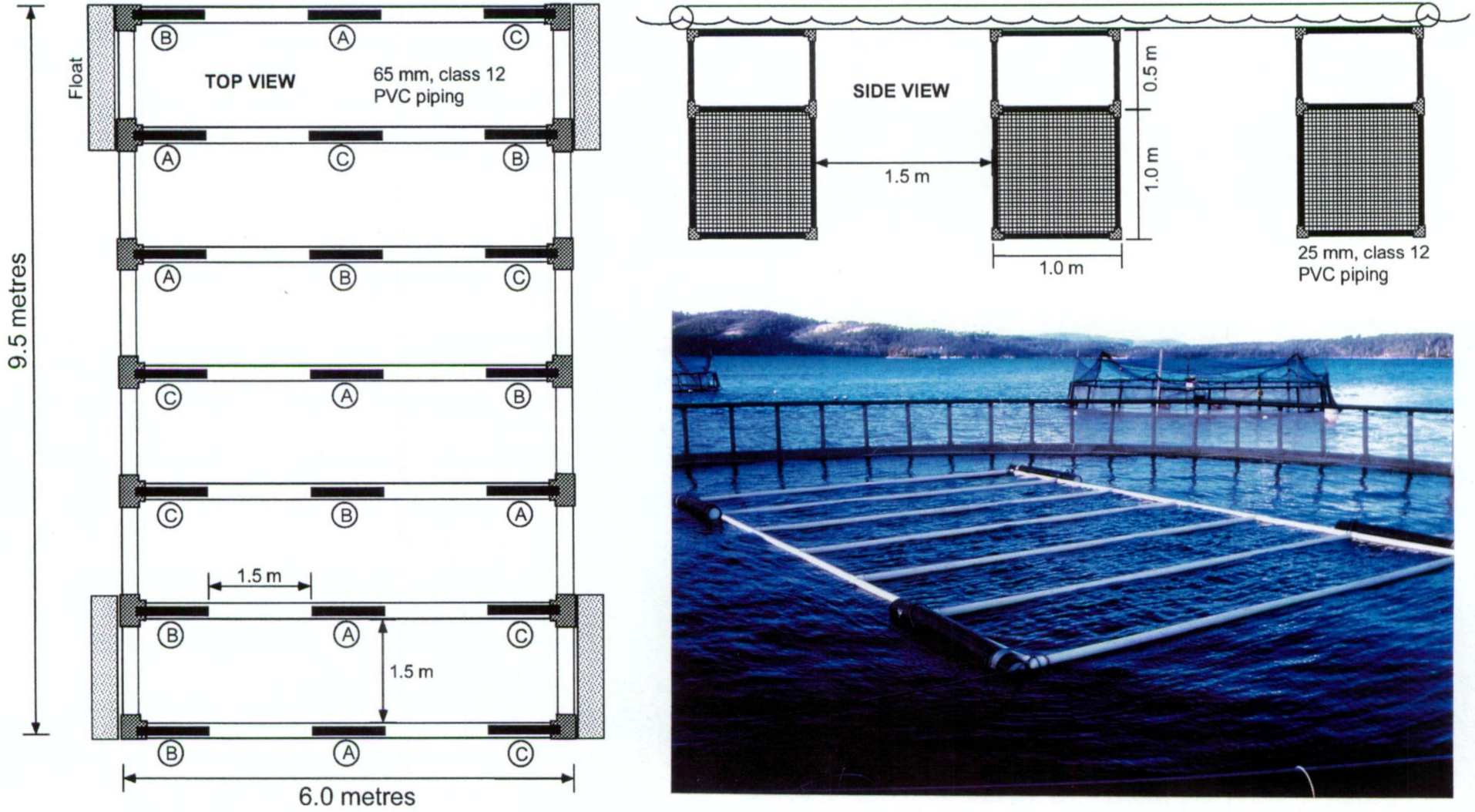


Figure 51. Raft and panel design for testing of silicone-coated netting. Panels were attached to the raft in a 1-way block design, with each of the 3 treatments (Ⓐ = black net; Ⓑ = white net; Ⓒ = silicone-coated net) positioned randomly within each of the 7 bars. Each panel held a 1 x 1 m (40 X 40 mesh holes) section of netting at 0.5 m to 1.5 m depth.

differences in fouling mass typically observed between the northern and southern sides of cages. The panels held the netting between 0.5 m and 1.5 m depth, so that netting was out of the turbulence of the surface waters where fouling development is generally atypical of most cage fouling. A 25 mm mesh net (regularly changed and cleaned) was attached to the cage to protect the panels from grazing by fish. Swain and Schultz (1996) reported that reduced fouling on silicone coatings may be partly attributed to the increased success of fouling removal by grazing fish.

Fouling development was monitored by wide-angle photography using a Nikonos-V camera with a 20 mm lens. Photographs were taken after 9 days immersion and then at approximately monthly intervals for 5 months.

4.3.2 Adhesion strength, composition and biomass of fouling

On January 27 (140 days immersion) the 9 panels (i.e. 3 replicates per net type) on the southernmost 3 rows were removed to quantify ease of fouling removal and fouling composition. The central 30 x 30 mesh-hole area was sampled from each panel (56% of the total area) and divided into four 15 X 15 mesh areas for ease of sampling. The panels were mounted horizontally and each side of each 15 X 15 mesh area cleaned with a water jet at a fixed distance above the net (30 cm) (Fig. 52). Constant water pressure was maintained throughout the trial (115 psi, delivering 0.33 l/sec) and each area was cleaned for 10 seconds. The panel mesh surrounding each sample area was masked by plastic sheeting during cleaning (Fig. 52B). Dislodged fouling was collected below the panel in a bucket and strained through 1 mm mesh. Fouling still attached to the net was removed with forceps. Samples were fixed with 10% formalin in sea water and stored at 4°C in the dark prior to analysis.

The total wet weight of remnant and dislodged fouling from each area was measured to ±0.0001 g after the excess water was drained from each sample (through 1 mm mesh). Each sample was then sorted into species, dried to a constant weight at 60°C, and the total mass for each species weighed to ±0.0001 g.

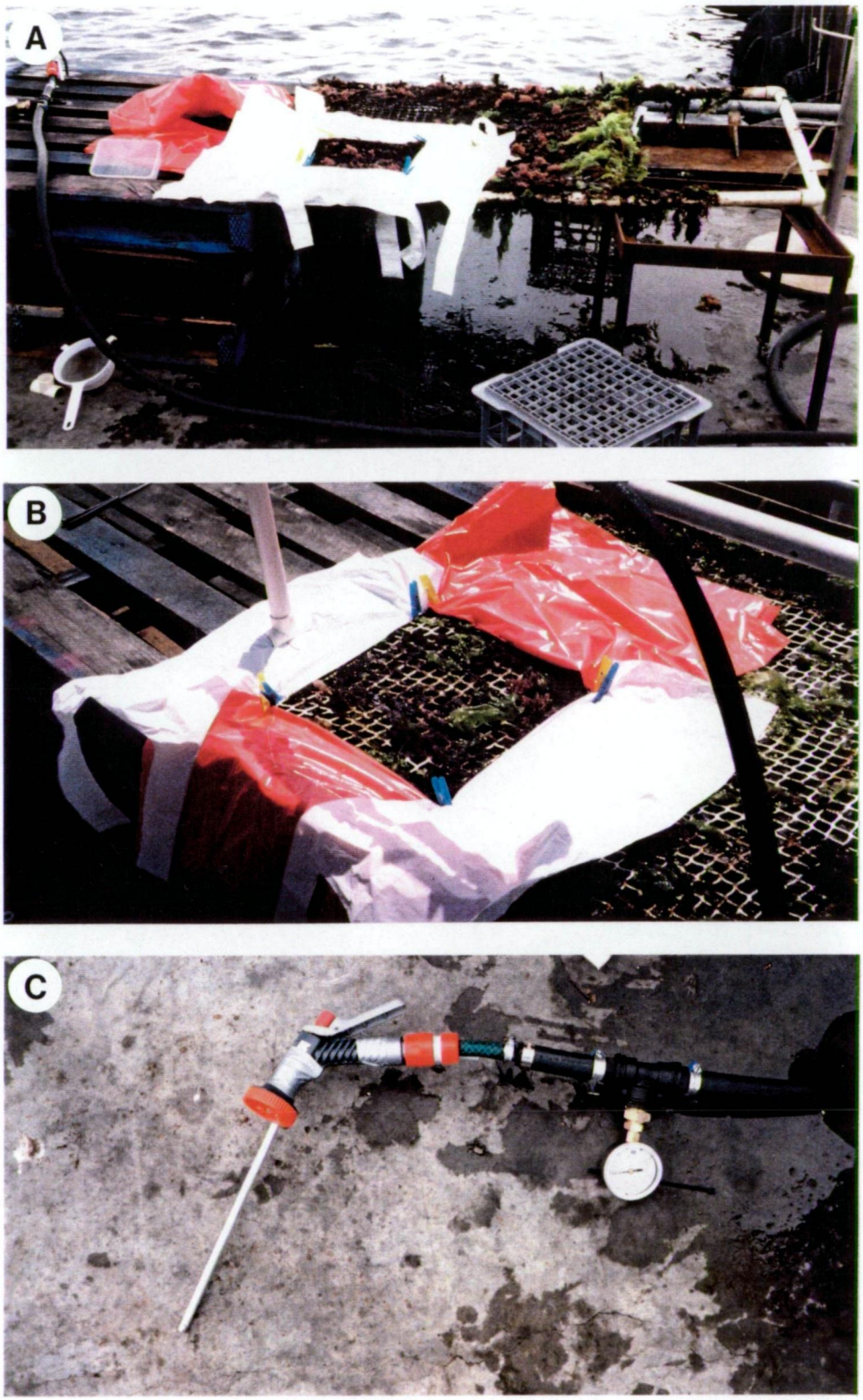


Figure 52. Cleaning head and panel setup for quantifying ease of fouling removal from silicone coated and uncoated netting. A: panels were mounted horizontally for sampling. B: the netting surrounding each sample area was masked with plastic sheeting during application of the water jet. C: the cleaning head.

4.3.3 *in situ* mechanical cleaning

On February 18 (163 days immersion) the remaining 12 panels were used to quantify ease of fouling removal with the prototype *in situ* cleaner (Fig. 30). Two rows on the raft were chosen randomly (from the four) and the 6 panels attached to the side of a salmon cage and cleaned. The netting was then removed from the 6 cleaned panels, and from the remaining 6 non-cleaned panels, and weighed to ±0.01 kg. Wide-angle underwater photographs were taken to record the level of fouling on each panel. The total wet weight of attached fouling was determined by subtracting the weight of clean wet netting (silicone coated = 0.75 kg/m^2; untreated net = 0.47 kg/m^2) from the gross wet weight.

4.3.4 Statistical analysis

The biomass per species, total attached fouling (algal, invertebrate and combined) and total dislodged material was compared between each netting type (black, white and silicone-coated) using one-way analysis of variance (ANOVA). Data shown to have significant differences were further analysed using Tukey's test. Data was analysed by ANOVA, rather than as a blocked design, because variation between blocks was not significant. Prior to ANOVA each data set was tested for homogeneity of variance (Cochran's test) and normality (using residuals, Shapiro-Wilk W test). Data that did not meet these assumptions were transformed to the common log prior to analysis. In cases where a zero occurred in a replicate(s) (eg. where a species was not found on each panel) all data was transformation by log (Y + 1) (Sokal & Rohlf, 1981).

Results from the trial with *in situ* mechanical cleaning were analysed by two-way ANOVA, using netting type and cleaning treatment as the two factors, followed by planned comparisons (LSD).

Analysis was conducted using the software JMP 3.1 (Macintosh).

4.4 Results

4.4.1 Fouling composition and biomass

After 9 days immersion all uncoated netting was heavily fouled by diatoms (Fig. 53). However, this type of fouling was weakly attached to the silicone and only masses of diatoms that completely encircled the mesh bars had remained. Fouling development proceeded with rapid growth of algae (primarily rhodophyceae) and by 110 days all untreated netting was heavily fouled (Fig. 54A). Fouling on the silicone panels showed a high degree of variability, and large clean areas of mesh appeared on all panels due to algal material sloughing from the surface (Fig. 54B). *Ulva rigida* was abundant on the silicone coating, but largely absent from the untreated nets. Solitary ascidians became the dominant fouling organism on untreated netting after 140 days, but were infrequent on the silicone (Fig. 55). At this time, areas free of fouling were still common on the silicone (Fig. 55C, 55D), but attached *U. rigida* plants had greatly increased in size (Fig. 56). The preferential colonisation of *U. rigida* on the silicone was clearly evident (Fig. 56).

After 140 days immersion the total wet biomass differed significantly between each type of netting (Fig. 57). White netting had the greatest total biomass and the silicone-coating the least. The difference in total fouling between the uncoated white and black netting resulted from a significantly greater algal mass on white netting (Fig. 58A). The invertebrate masses on both types of uncoated net were nearly identical (Fig. 59A). The silicone coating had far less invertebrate fouling than the uncoated netting (Fig. 59A), but a similar level of algal fouling to the white netting (Fig. 58A).

Significant differences in the composition of the algal and invertebrate communities were identified between the 3 netting types (Figs 58B, 59B, Table 7). The lower algal mass on black netting largely resulted from a significantly greater *U. rigida* mass on the white netting and silicone coating (Table 7). As a percentage of the total algal biomass, *U. rigida* was the dominant species on both the white netting (37%) and the silicone coating (63%) (Fig 58B). However, although the black netting had the least algae, it was fouled by a significantly greater mass of *Griffithsia monilis* (Table 7) which comprised 30% of the total algal mass (Fig. 58B). With the exception of *G. monilis* all algal species occurred in greater biomass on the white than black netting (although a statistically significant difference was found for only *U. rigida* and *Champia affinis*). Similarly, the majority of algal species were more abundant on the white netting than the silicone coating. However, a similar total mass occurred on these net types due to the

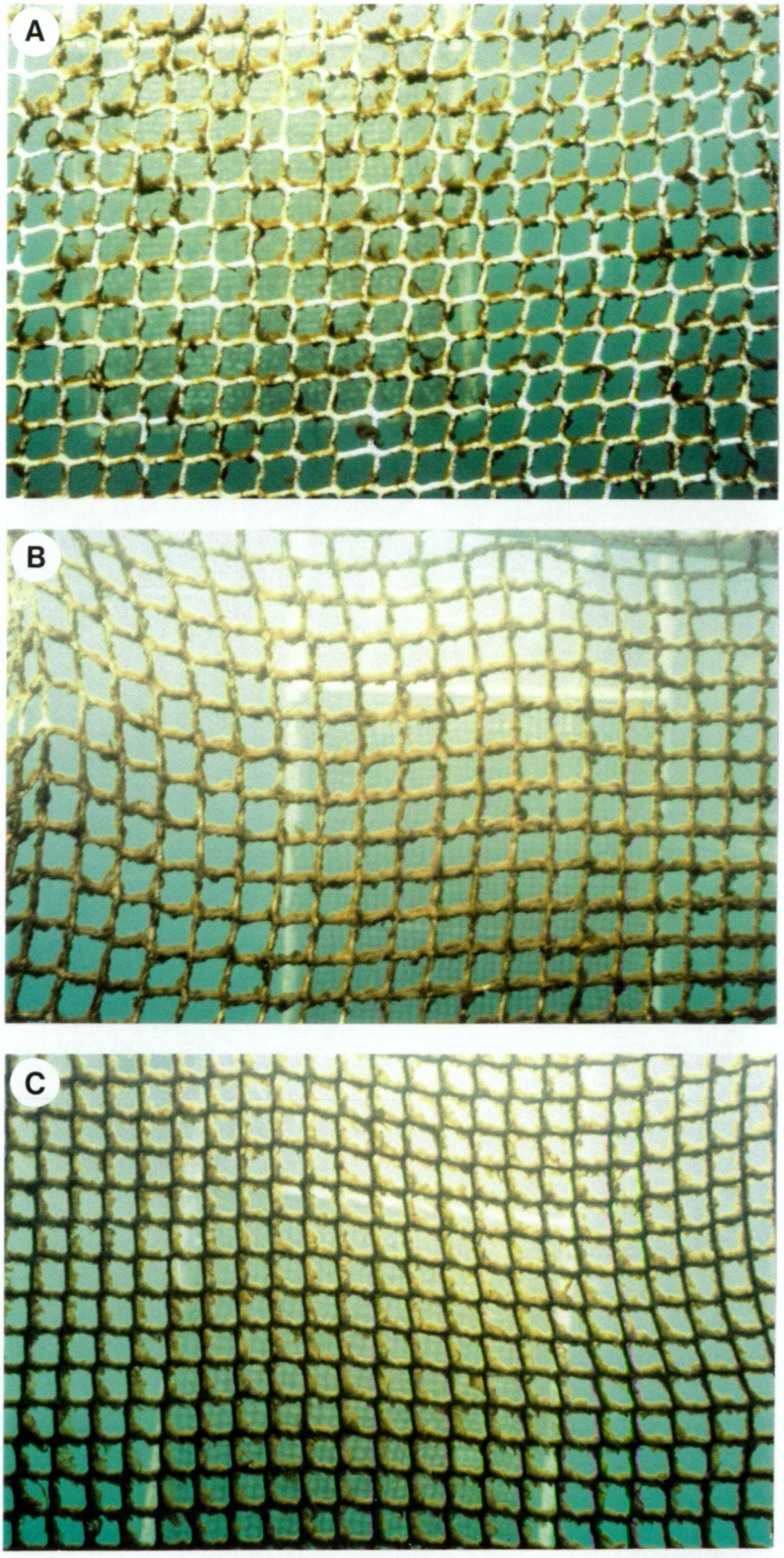


Figure 53. Diatom growth on the netting panels after 9 days immersion. A: Colonisation of the silicone panel was patchy and only those masses that completely encircled the bars remained attached. B and C: diatom fouling on white and black netting evenly covered all the mesh.

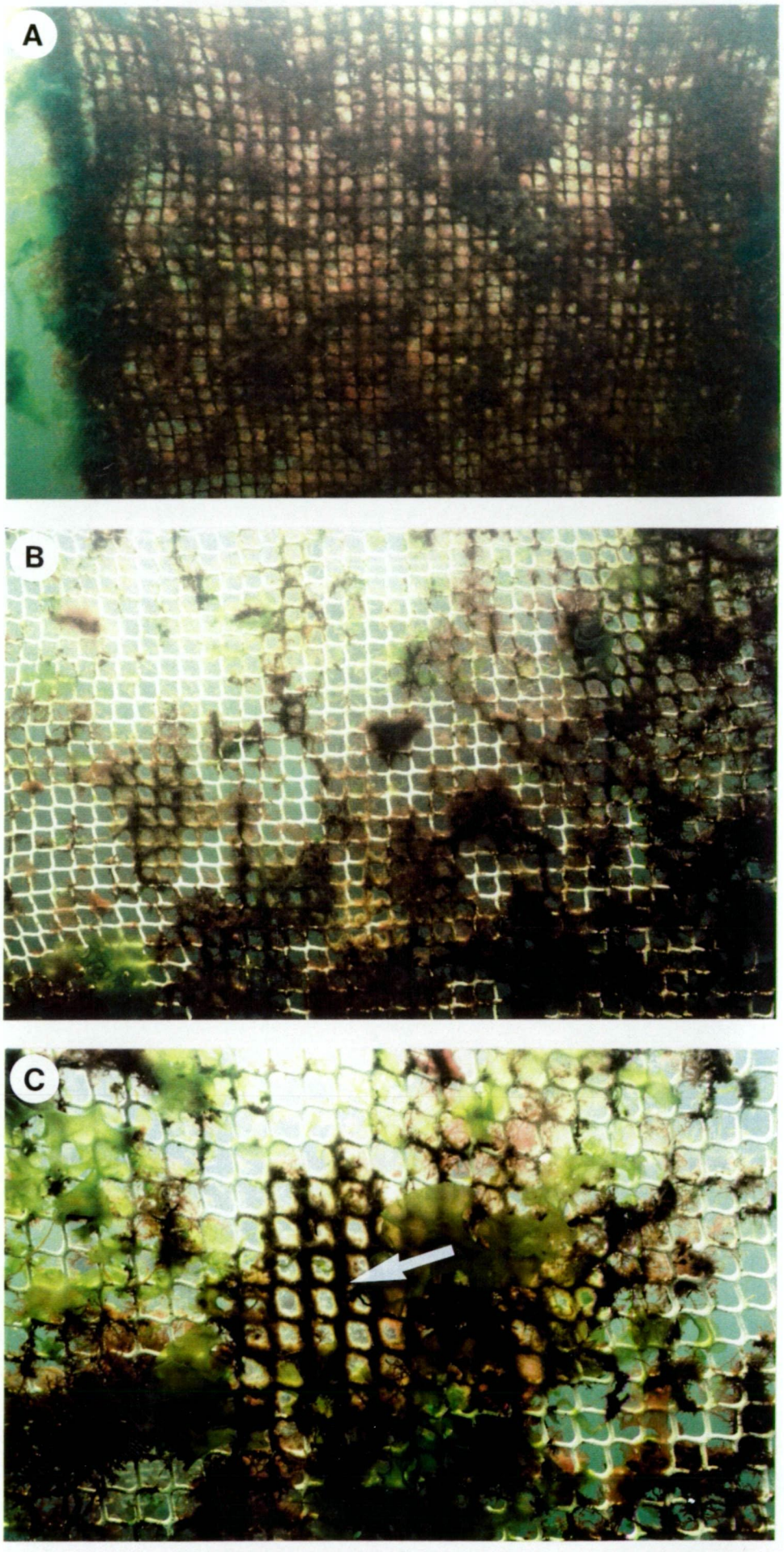


Figure 54. Fouling growth after 110 days immersion. A: panels of uncoated netting were heavily fouled by algae. B: fouling growth on the silicone coating was patchy and many areas were clean because fouling had sloughed from the surface. C: *Ulva rigida* was abundant on the silicone coating. The centre of the photograph shows a colonial ascidian (arrow).

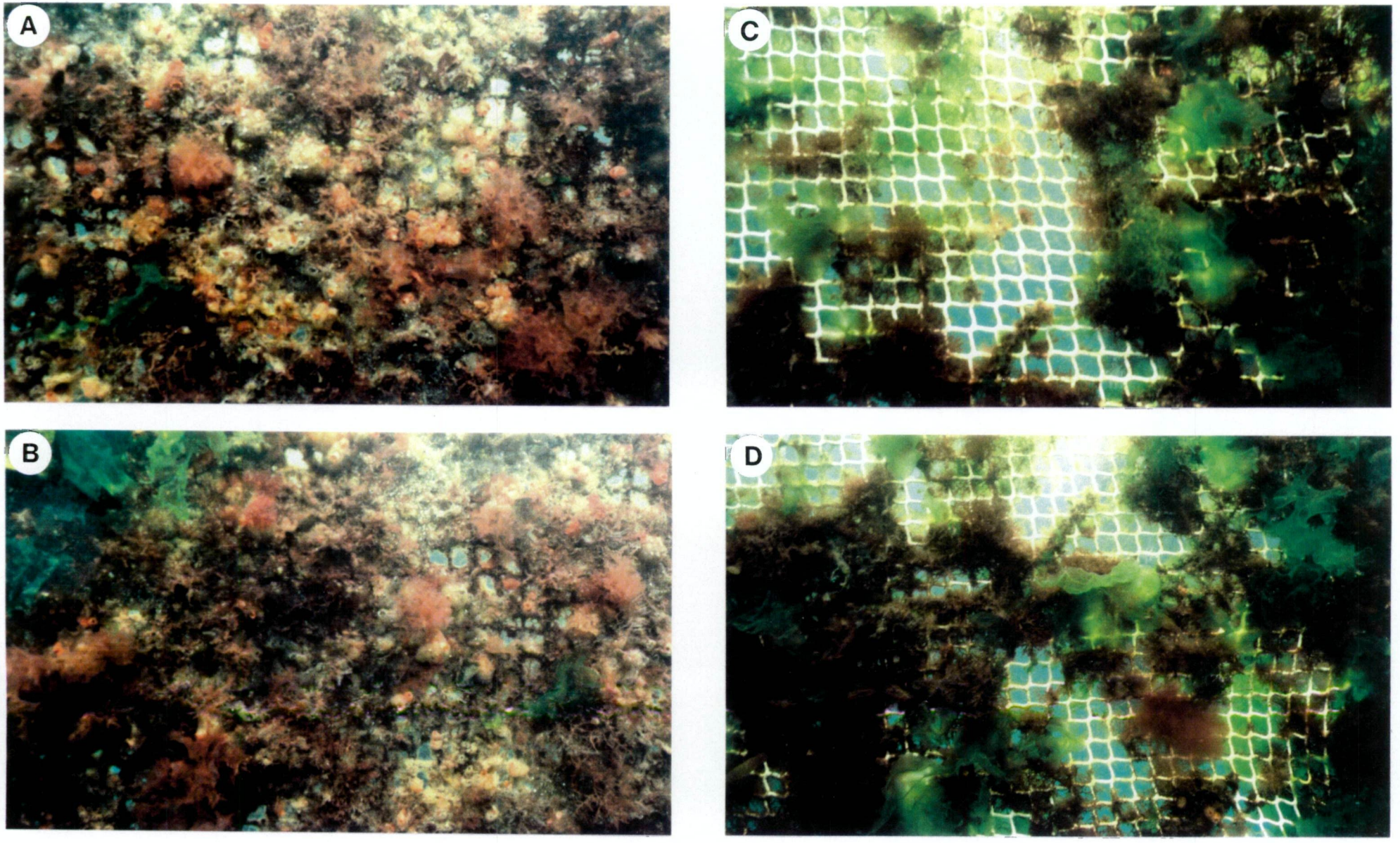


Figure 55. Fouling growth after 140 days immersion. A, B: the uncoated netting was heavily fouled by algae and solitary ascidians. C, D: the silicone-coated netting had less fouling than the uncoated nets, and very few ascidians were present. *Ulva rigida* was abundant on the silicone coating.

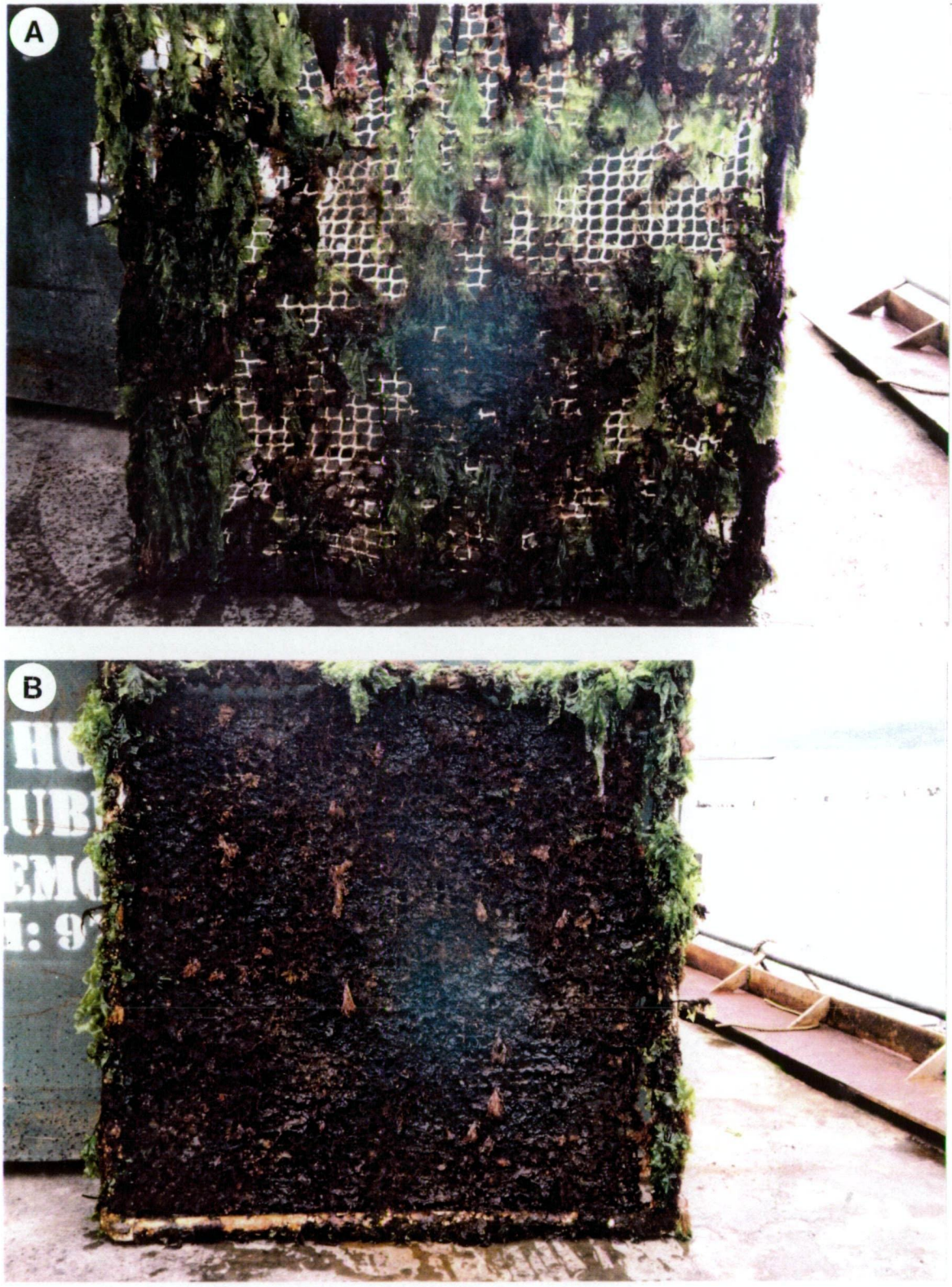


Figure 56. Fouling panels removed from the water after 140 days immersion. A: silicone-coated netting predominantly fouled by *Ulva rigida*. Some areas of the mesh were free of fouling. B: the uncoated nets were heavily fouled and the majority of mesh was completely occluded.

greater quantities of *U. rigida*, *Polysiphonia brodiaei* and *Lomentaria australis* on the silicone.

The composition of the invertebrate communities was nearly identical for the white and black uncoated netting (Fig. 59B) and no significant differences between the mass of each species was found for these net types (Table 7). Greater than 75% of the biomass on both types of uncoated netting was comprised of solitary ascidians (Fig. 59B). The silicone coating was fouled by a significantly lower invertebrate mass (Fig. 59A) and solitary ascidians comprised only 26% of the community (Fig. 59B). The total dry mass of solitary ascidians weighed 17.0 g (dry weight/0.25 m^2) on each of the uncoated netting types, but only 1.3 g on the silicone coating. The dominant invertebrate on the silicone coating was a colonial ascidian (eg. Fig. 54C), but its mean biomass was no greater than that found on the uncoated nets (Table 7).

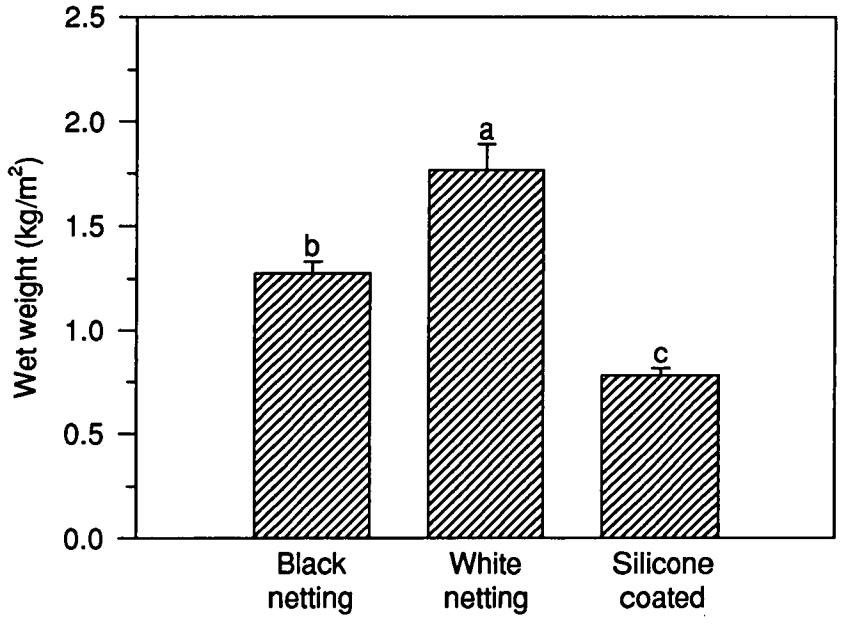


Figure 57. Total wet fouling biomass on netting panels after 140 days immersion. Each column represents the mean of 3 panels. Bars = standard error. The biomass was found to differ significantly between each net type ($P < 0.05$, Tukey's test).

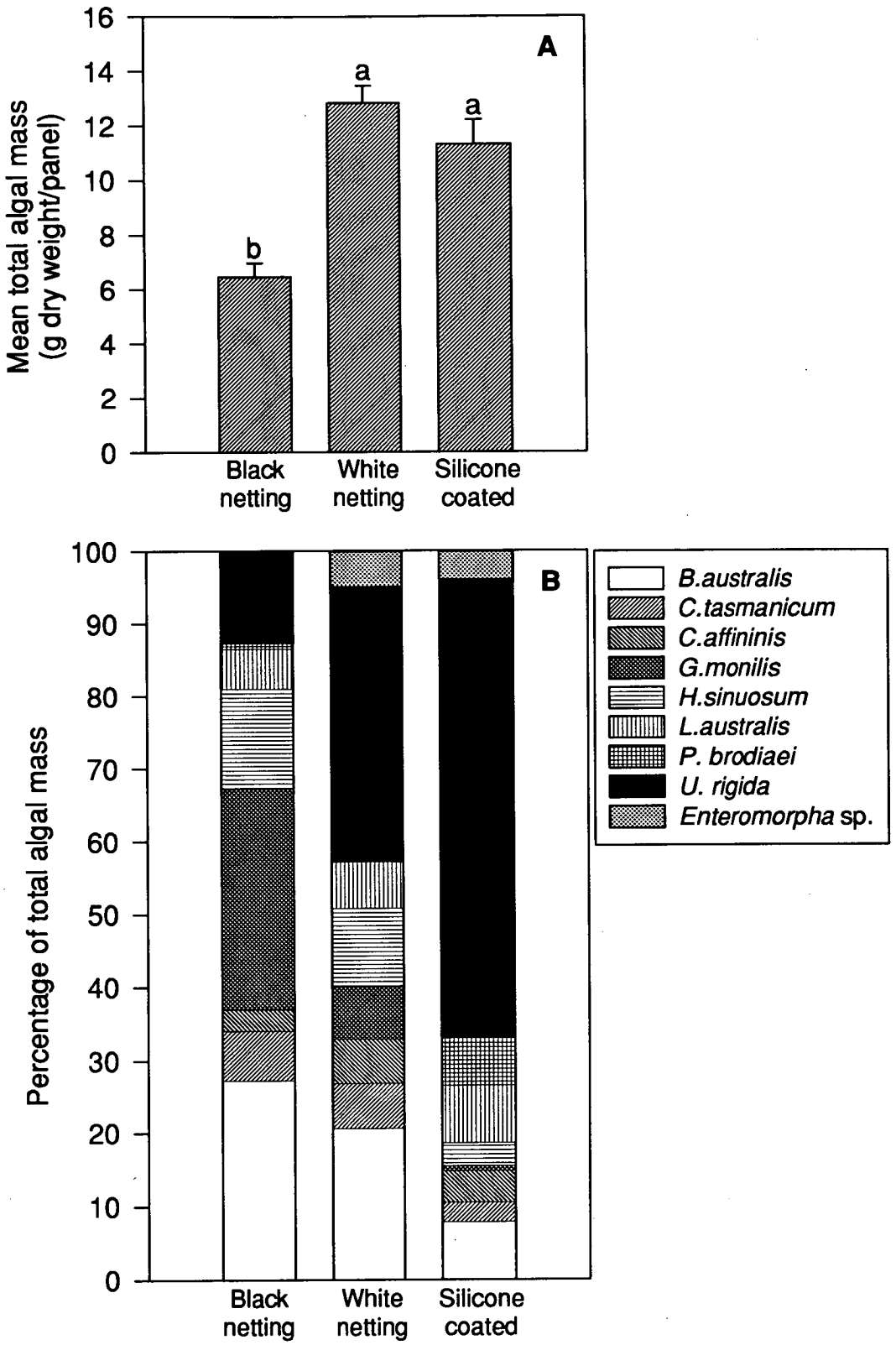


Figure 58. Biomass and composition of the algal community on panels immersed for 140 days. A: total algal biomass, dry weight. Each column represents the mean of 3 panels. Bars = standard error. Columns sharing the same letter are not significantly different ($P > 0.05$, Tukey's test). B: relative abundance of algal species on each type of netting. The proportion of each species represents the percentage of total dry algal mass.

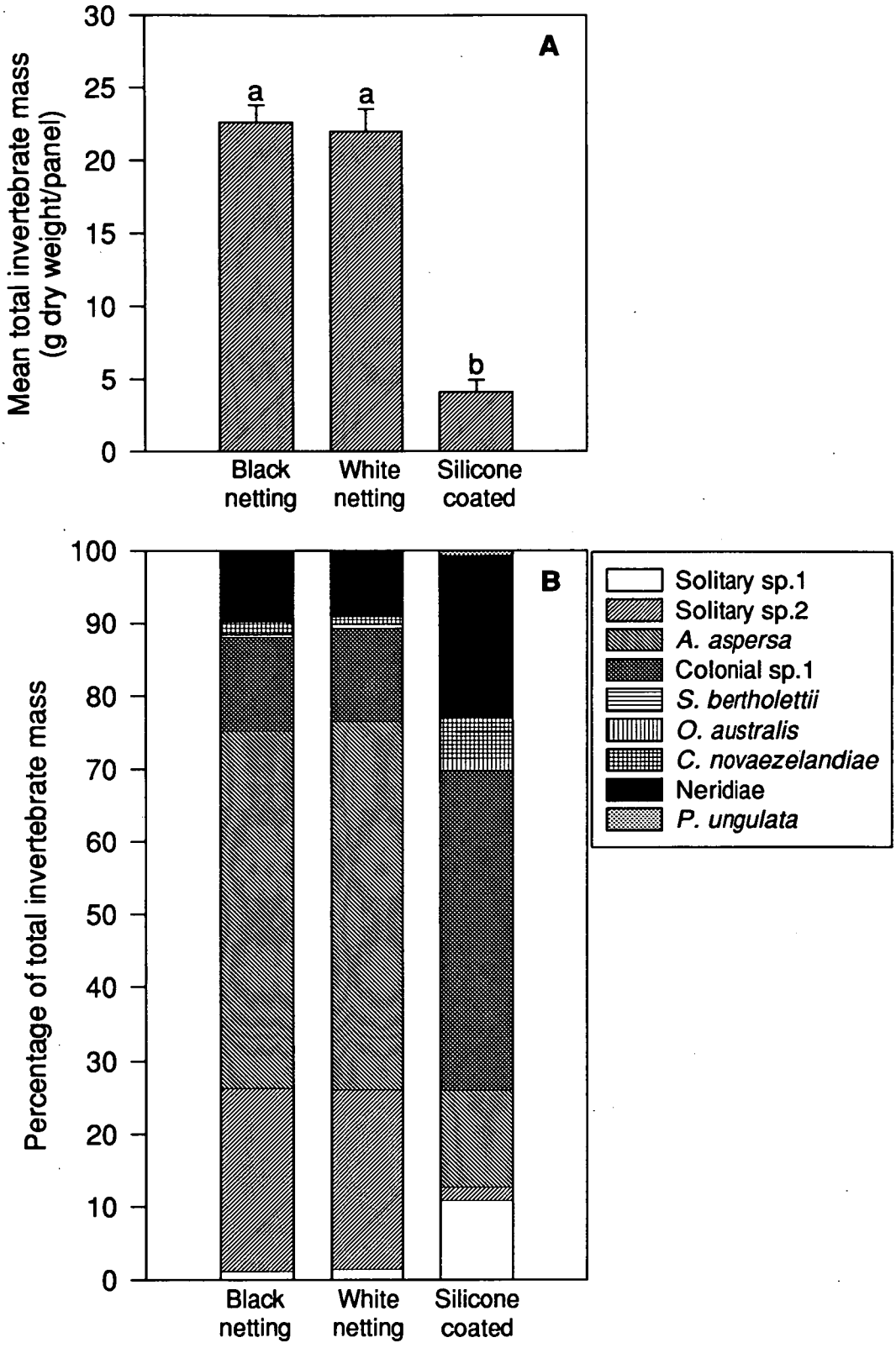


Figure 59. Biomass and composition of the invertebrate community on panels immersed for 140 days. A: total invertebrate biomass, dry weight. Each column represents the mean of 3 panels. Bars = standard error. Columns sharing the same letter are not significantly different ($P > 0.05$, Tukey's test). B: relative abundance of invertebrate species on each type of netting. The proportion of each species represents the percentage of total dry invertebrate mass.

Table 7. Mean dry weight (g) for each species per sample area (g/0.25m^2). 140 days immersion.

Taxa	Black netting	White netting	Silicone	Differences[a]
Algae				
Rhodophyceae				
Brongniartella australis	1.828 ± 0.570	2.669 ± 0.901	0.914 ± 0.262	
Ceramium tasmanicum	0.421 ± 0.126	0.850 ± 0.446	0.295 ± 0.132	
Champia affinis	0.181 ± 0.036	0.781 ± 0.159	0.485 ± 0.063	b: a: a,b
Griffithsia monilis	1.883 ± 0.423	0.916 ± 0.324	0.077 ± 0.036	a: a: b
Haraldiophyllum sinuosum	0.894 ± 0.163	1.374 ± 0.081	0.380 ± 0.196	a,b: a: b
Lomentaria australis	0.378 ± 0.157	0.787 ± 0.265	0.903 ± 0.099	
Polysiphonia brodiaei	0.040 ± 0.024	0.048 ± 0.034	0.740 ± 0.080	b: b: a
Chlorophyceae				
Enteromorpha sp.	0.019 ± 0.019	0.707 ± 0.704	0.439 ± 0.342	
Ulva rigida	0.795 ± 0.295	4.713 ± 1.314	7.084 ± 0.727	b: a: a
Invertebrates				
sessile				
Ascidiacea				
Solitary sp. 1	0.269 ± 0.140	0.333 ± 0.256	0.540 ± 0.536	
Solitary sp. 2	5.651 ± 0.142	5.363 ± 0.711	0.086 ± 0.049	a: a: b
Ascidiella aspersa	11.167 ± 1.478	11.211 ± 1.861	0.644 ± 0.314	a: a: b
Colonial sp. 1	2.866 ± 0.715	2.745 ± 0.536	1.743 ± 0.759	
Bryozoa				
Scrupocellaria bertholettii	0.082 ± 0.043	0.125 ± 0.125	0.008 ± 0.008	
Hydrozoa				
Obelia australis	0.012 ± 0.006	0.015 ± 0.006	0.056 ± 0.018	
non-sessile				
Decapoda				
Cancer novaezelandiae	0.385 ± 0.196	0.218 ± 0.036	0.220 ± 0.065	
Polychaeta				
Nereidae	2.153 ± 0.086	1.924 ± 0.189	0.752 ± 0.147	a: a: b
Isopoda				
Paridotea ungulata	0.031 ± 0.016	0.045 ± 0.024	0.035 ± 0.032	
Amphipoda				
Caprella sp.	0	0	0.007 ± 0.007	

[a] Letters are given where means (n=3) differ significantly between netting types, and are allocated in the order *black netting: white netting: silicone*. Letters are allocated in descending order, from the highest to lowest mean. Netting types sharing the same letter are not significantly different ($P > 0.05$, Tukey's test).

4.4.2 Ease of fouling removal

The application of a low-pressure water jet removed a significantly greater mass of fouling from the silicone-coated netting than the uncoated netting (Fig. 60). As a percent of the total initial biomass, 15.3% of the fouling was removed from the silicone coating, but only 3.0% and 3.6% was removed from the white and black netting, respectively. The increased ease of fouling removal from the silicone coating is clearly shown in Figs. 61-62. However, although fouling was easily dislodged from this surface a greater level of removal was prevented because *U. rigida* became caught over the mesh and some solitary ascidians were too large to fit through the mesh (eg. Fig. 61B). After cleaning, the remnant fouling was easily collected from the silicone coating by gently wiping the surface. In comparison, it typically took 15 minutes to pull residual fouling from the uncoated nets.

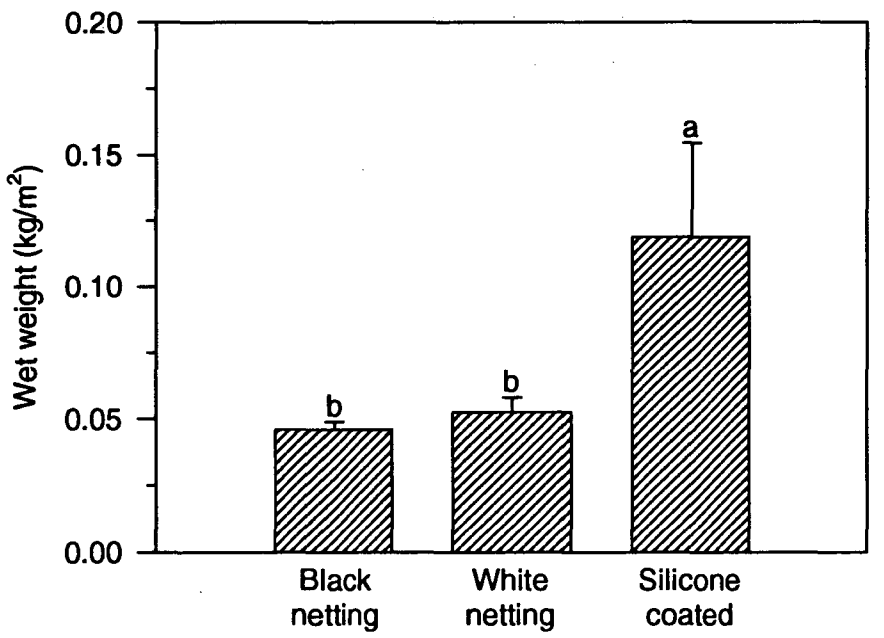


Figure 60. Total wet biomass dislodged from each type of netting after cleaning with a medium-pressure water hose. Each column represents the mean of 3 panels. Bars = standard error. Columns sharing the same letter are not significantly different ($P > 0.05$, Tukey's test).

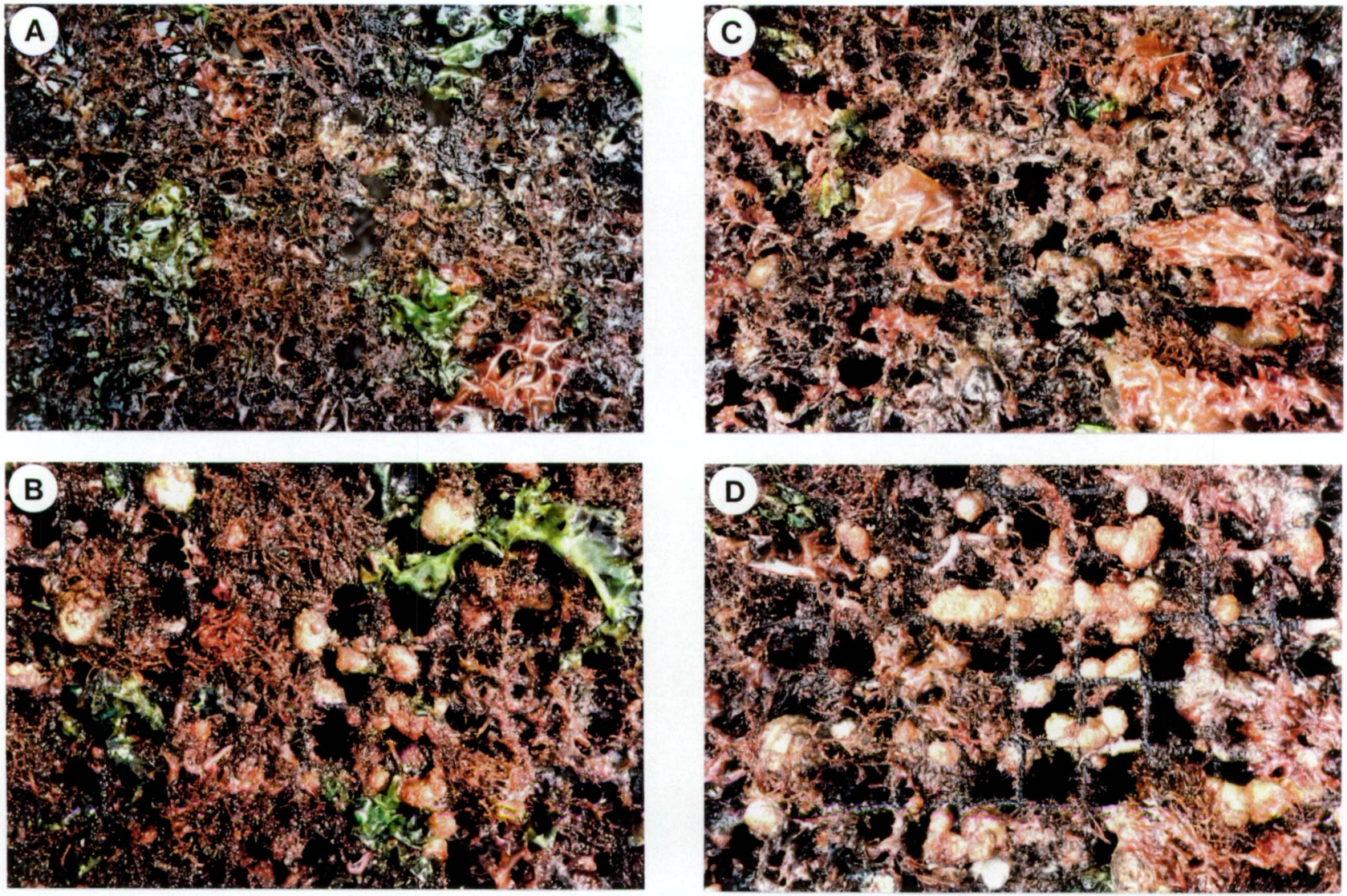


Figure 61. Uncoated netting before and after cleaning with the water jet. A: site 1 before. B: site 1 after. C: site 2 before. D: site 2 after. Only minor quantities of fouling were removed, and solitary ascidia were unaffected.

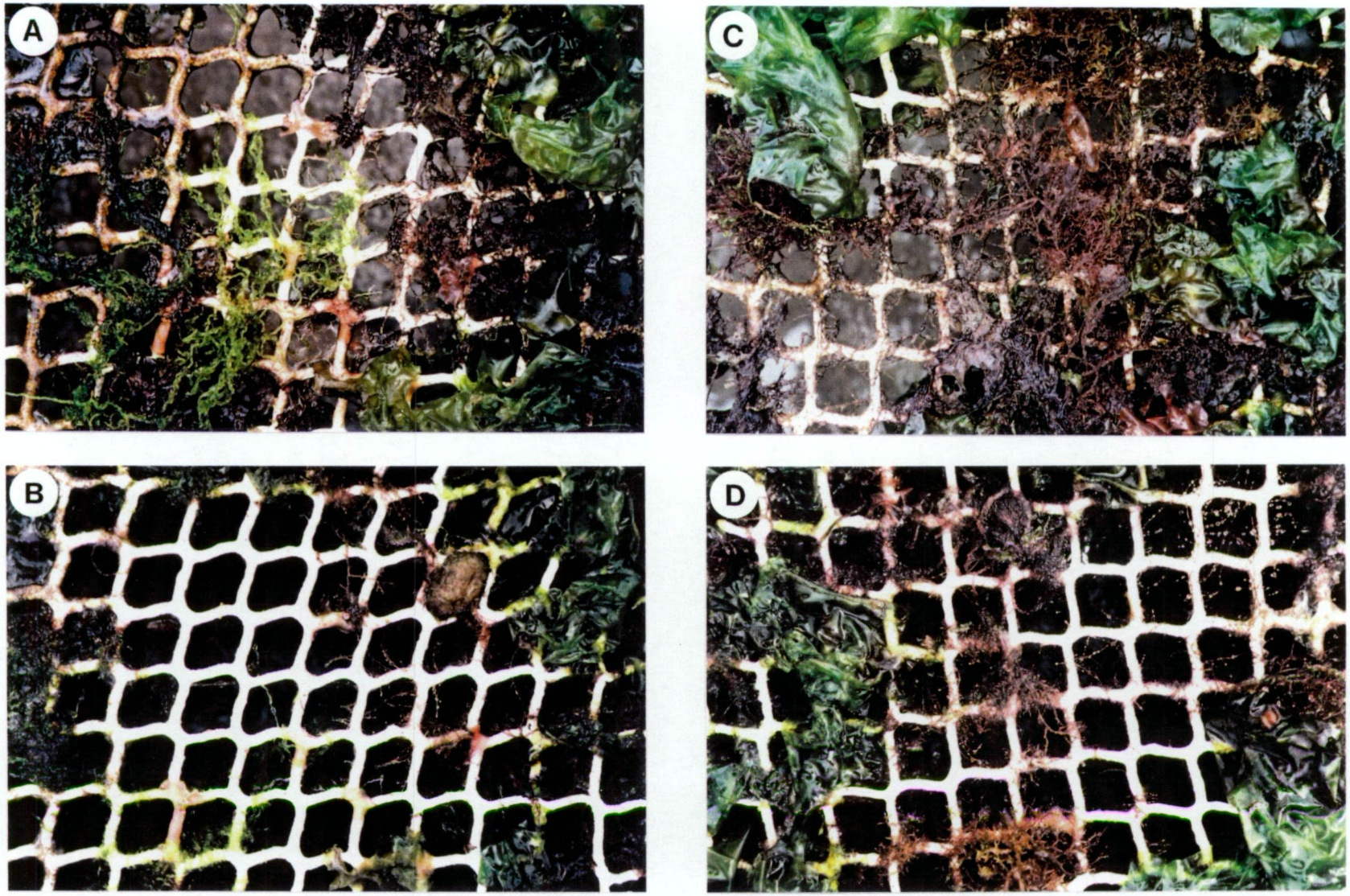


Figure 62. Silicone-coated netting before and after cleaning with the water jet. A: site 1 before. B: site 1 after. C: site 2 before. D: site 2 after. Fouling was easily dislodged from the silicone coating, but *Ulva* often remained because it was caught over the mesh.

Compared with uncoated netting, a significantly greater percentage of both the algal and invertebrate community was removed from the silicone coating (Figs 63A, 64A). Of the algae, *U. rigida* comprised the greatest proportion of the dislodged algal mass from each netting type (Fig. 63B). Of the invertebrates, the majority of dislodged biomass comprised non-sessile species (Fig. 64B, Table 8). Although solitary ascidians comprised the majority of invertebrate biomass on uncoated netting (Table 7), very little of this fouling was removed (Fig. 64B, Table 8). However, the few ascidians that were present on the silicone were easily dislodged (Table 8), and comprised greater than 20% of the total dislodged invertebrate mass.

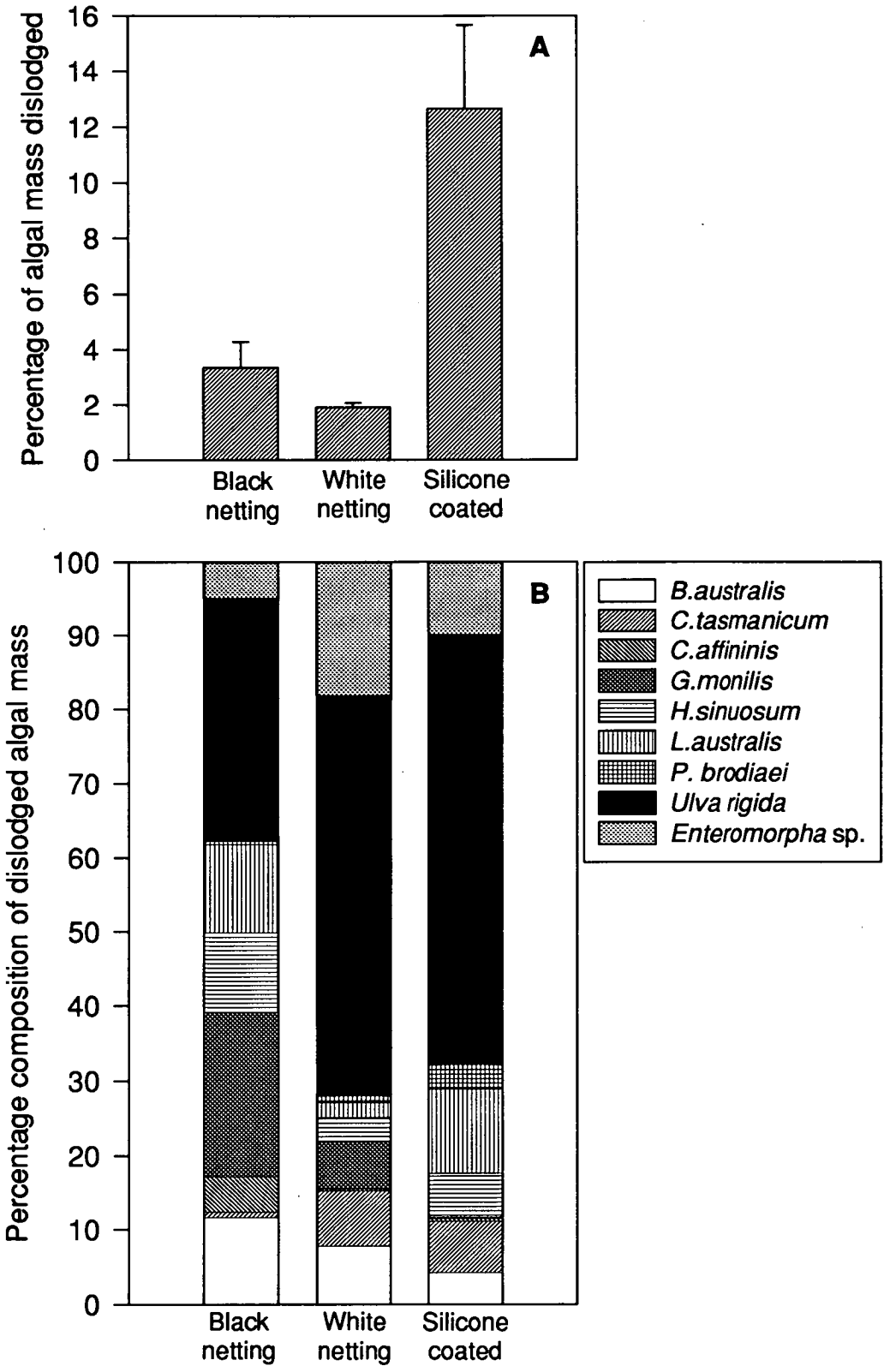


Figure 63. Biomass and composition of the algal material dislodged from the netting after cleaning. A: total algal biomass, dry weight. Each column represents the mean of 3 panels. Bars = standard error. B: relative abundance of algal species in dislodged material. The proportion of each species represents the percentage of total dry algal mass.

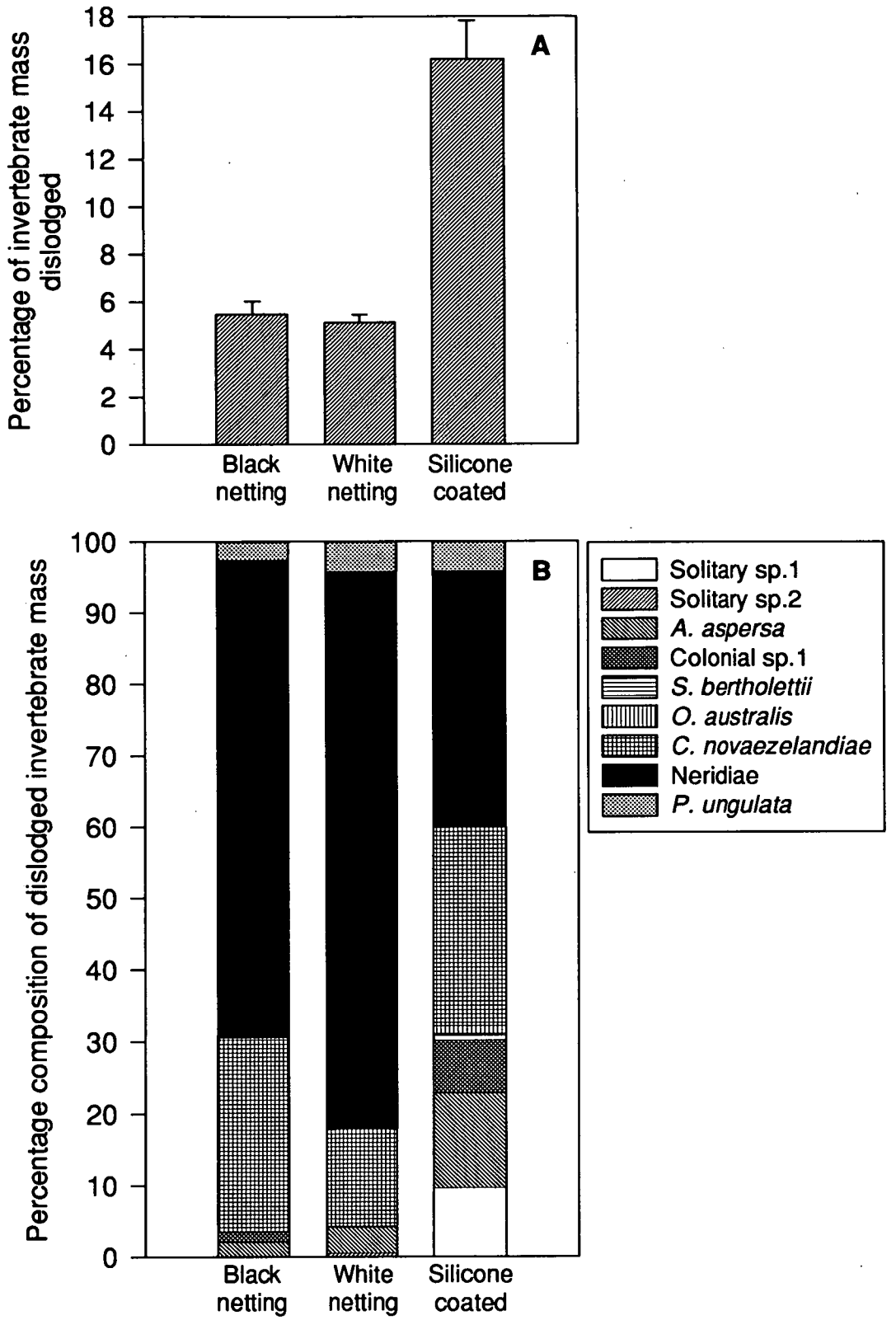


Figure 64. Biomass and composition of the invertebrate material dislodged from the netting after cleaning. A: total invertebrate biomass, dry weight. Each column represents the mean of 3 panels. Bars = standard error. B: relative abundance of invertebrate species in dislodged material. The proportion of each species represents the percentage of total dry invertebrate mass.

Table 8. Percent of each species dislodged from the netting by a medium-pressure water jet*.

Taxa	Black netting	White netting	Silicone
Algae			
Rhodophyceae			
Brongniartella australis	1.3	0.7	3.5
Ceramium tasmanicum	0.6	1.8	18.8
Champia affinis	3.7	0.8	0.5
Griffithsia monilis	0.9	1.9	4.7
Haraldiophyllum sinuosum	2.9	0.5	20.5
Lomentaria australis	11.3	0.7	22.7
Polysiphonia brodiaei	3.8	7.5	7.1
Chlorophyceae			
Enteromorpha sp.	100.0	7.1	28.4
Ulva rigida	10.0	2.7	13.6
Invertebrates			
sessile			
Ascidiae			
Solitary sp. 1	0.0	0.0	17.2
Solitary sp. 2	0.0	0.1	0.0
Ascidiella aspersa	0.0	0.4	10.3
Colonial sp. 1	0.5	0.1	3.7
Bryozoa			
Scrupocellaria bertholettii	0.0	0.0	100.0
Hydrozoa			
Obelia australis	0.3	0.0	9.2
non-sessile			
Decapoda			
Cancer novaezelandiae	99.3	69.3	84.3
Polychaetes			
Nereidae	35.8	44.6	24.4
Isopoda			
Paridotea ungulata	100.0	100.0	100.0
Amphipoda			
Caprella sp.	.	.	100.0

* 100% = all removed

4.4.3 *in situ* mechanical cleaning

After 163 days immersion the total wet biomass on the untreated nets weighed greater than 7.5 kg/m^2 (Fig. 65). This large increase from 140 days (Fig. 57) resulted from the rapid growth of solitary ascidians (Fig. 66), and the large mass primarily represented the water contained within their test. The increase in fouling biomass on the silicone resulted largely from growth of attached algae.

Cleaning with the prototype *in situ* cleaner (Fig. 30) resulted in a significant ($P < 0.05$) decrease in mass for both types of uncoated net. Similarly, cleaning decreased the fouling level on the silicone coating, but the low replication limited statistical power for this comparison (Power = 0.23) and a statistically significant difference was not found ($P = 0.17$). However, the cleaner was successful in removing fouling from the silicone coating (Fig. 67) and the mean fouling biomass was nearly halved after cleaning (Fig. 65).

On the uncoated netting the cleaner was observed to remove lengthy algal material and soft colonial ascidians. However, although severe scrubbing of the large solitary ascidians occurred, their great adhesion strength prevented any removal (Fig. 67). The poor adhesion strength of all material on the silicone resulted in removal of much of the fouling and, after cleaning, the majority of silicone coated netting had limited mesh occlusion (Fig. 67).

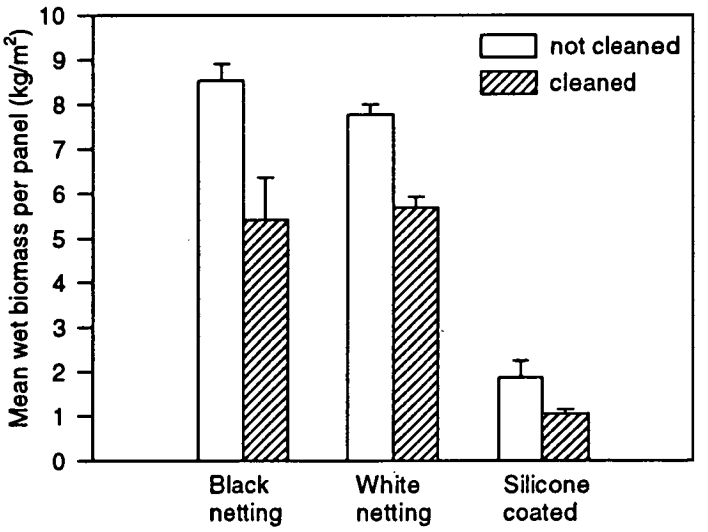


Figure 65. Total fouling mass on cleaned and uncleaned panels of different netting types. Cleaning was conducted with the prototype *in situ* net cleaner. Each column represents the mean of 2 samples. Bars = standard error.

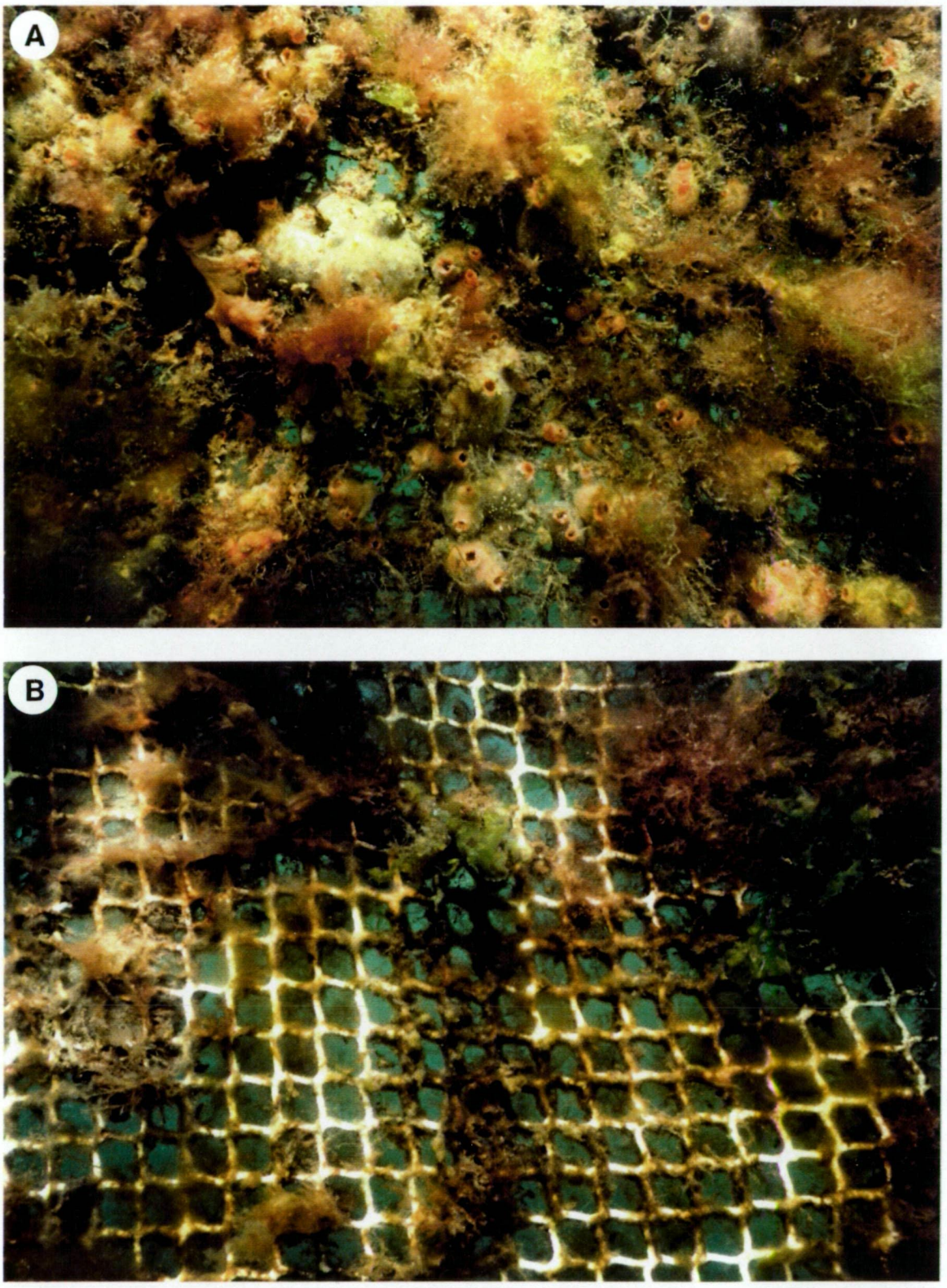


Figure 66. Fouling panels after 163 days immersion. A: the uncoated netting was heavily fouled by algae and solitary ascidia. B: the silicone-coated netting had some large fouling algae, but few ascidia and many areas had minimal occlusion.

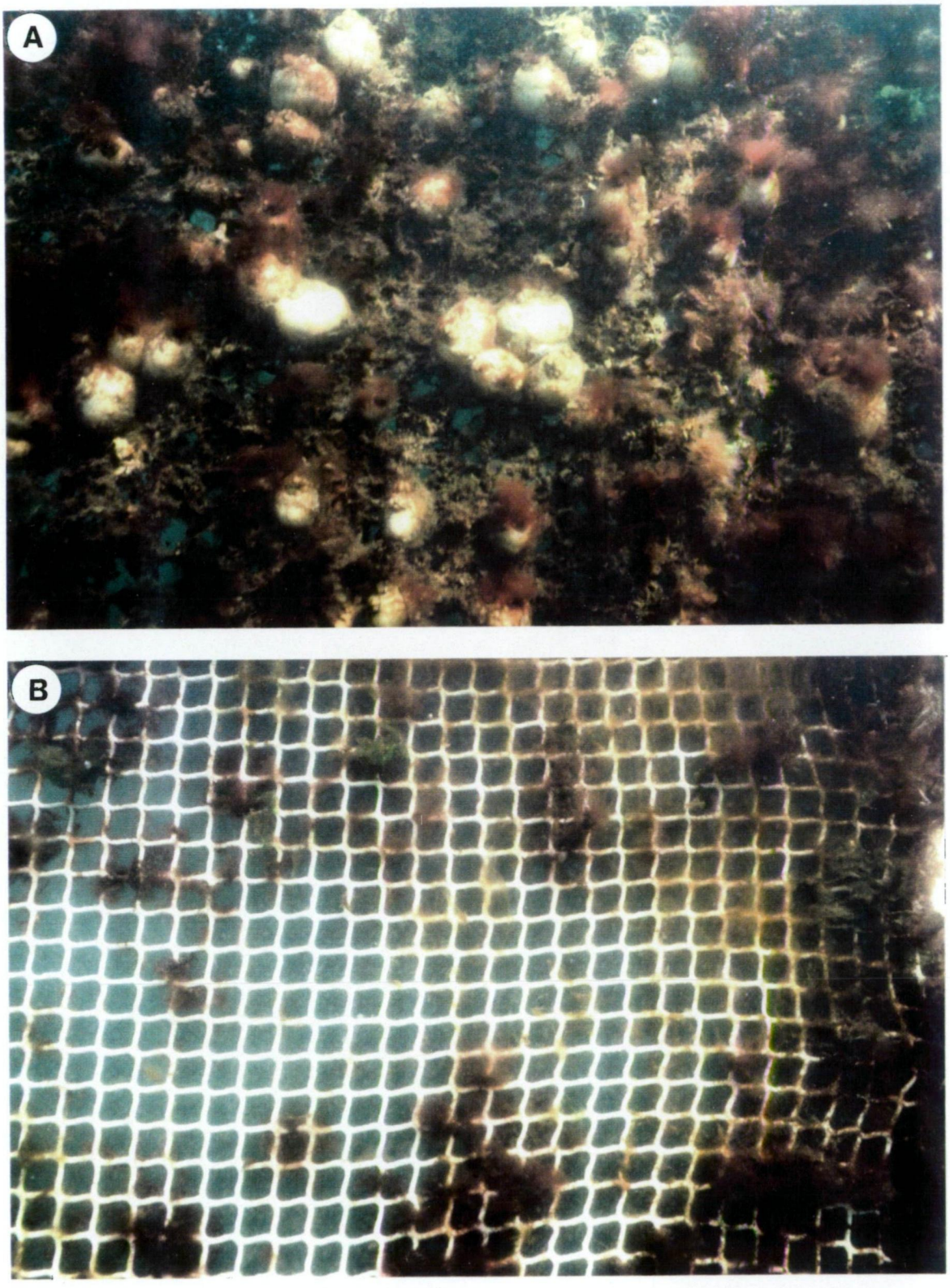


Figure 67. Panels after cleaning with the prototype *in situ* cleaner. A: some of the algal fouling was removed from the uncoated netting, but the large solitary ascidians remained attached. B: the majority of fouling was easily removed from the silicone coating.

4.5 Discussion

The primary aim of this study was to compare silicone-coated netting with uncoated netting in terms of fouling development and ease of fouling removal. Because the coating was white, the control for the experiment was white netting. However, black netting is the industry standard and panels of black netting were included to identify if colour alone could affect fouling development. The current industry preference for black nets is to reduce the visual impact of farms on the environment. However, white netting is easier to see during underwater net inspections and fish transferred to white nets prior to harvest change colour to a lighter, more marketable silver.

Surface colour has previously been found to affect the rate and composition of fouling communities (eg. Dahlem *et al.*, 1984). Larvae of many fouling invertebrates demonstrate a phototactic response during settlement (Crisp, 1974, 1984; Svane & Dolmer, 1995) and invertebrate fouling is more severe on darker surfaces (Dahlem, *et al.*, 1984; Henschel *et al.*, 1990). Zoospores of common fouling algae such as *Ectocarpus* and *Enteromorpha* react to light intensity (Fletcher *et al.*, 1984b), but have shown preference for lighter conditions (Christie & Shaw, 1968; Christie, 1972). In the present study, colour significantly affected algal fouling (Fig. 58) but not invertebrate fouling (Fig. 59). The change in algal growth is therefore not explained by invertebrate competition, but by increased algal settlement and/or growth on white surfaces. The greater abundance of *U. rigida* on the white netting compared to black netting probably resulted from an initial settlement preference as spores of green algae are motile and actively select suitable surfaces (Fletcher, 1980). The greater abundance of red algae maybe a result of increased light availability from reflection off the white surface rather than settlement preference, because spores of red algae are non motile and settlement is a passive process (Fletcher, 1980). Greater light availability would also explain the rapid growth of *U. rigida* on the white netting. The greater mass of the red alga *Griffithsia monolis* on black netting presumably occurred because there was less competition from other algal species.

The composition of the fouling community on the silicone coating is also likely to demonstrate preferential settlement and the effects of light reflection and reduced competition. However, the significantly greater biomass of *U. rigida* and *Polysiphonia brodiaei*, but relatively low abundance of other species also suggests a difference in attachment strength. The successful attachment of algal rhizoids results from physical interactions (eg. penetration of crevices) and/or chemical interactions (through adhesive mucilage) with the substratum, and is affected by the surface composition (chemical),

surface energy and surface texture (Fletcher *et al.*, 1984a; Roscoe & Walker, 1995). The smoothness of the silicone coating may have been unsuitable for some species as algal colonisation is correlated with surface roughness (Harlin & Lindbergh, 1977) and species such as *Laminaria digitata* primarily attach by mechanical interaction with substrate contours and crevices (Roscoe & Walker, 1995). Successful attachment of some species may also have been prevented because low surface energy can induce changes in rhizoid morphology (Marsland, 1975; Fletcher *et al.* 1984a). Two distinct forms of rhizoid are produced by algae: a compact disc-like structure of short, highly branched filaments and a loose structure of long outwardly-spreading free filaments (Fletcher *et al.*, 1984a, b). The former morphology is often associated with rough surfaces and results in significantly greater surface contact and adhesion than the elongate rhizoids, which are more common on smooth surfaces (Fletcher *et al.*, 1984a, b). Many common fouling algae, including *Enteromorpha intestinalis*, *Ulva lactuca*, *Bangia atropourea*, *Laminaria saccharina* develop well attached disc-like rhizoids on high energy surfaces, but weakly attached elongate rhizoids on silicone coatings (Marsland, 1975; Fletcher *et al.*, 1984a; Callow *et al.*, 1988).

Rhizoid morphology, and their area of surface contact, is unlikely to fully explain significant differences in adhesive strength for many species (Fletcher *et al.*, 1984a). Furthermore, not all algae show the same response to surface energy; the fouling algae *Polysiphonia urceolata* and *P. broadiaei* produce disc-like rhizoids on low energy surfaces and filamentous rhizoids on high energy surfaces, with little adhesive difference between each type (Fletcher, 1984a). The greater ability for some organisms to adhere to low energy surfaces may occur because their adhesives are complex enough to adhere to a wide range of surfaces (Swain *et al.*, 1992; Becker, 1993) or because they can alter adhesive composition in response to substrate surface energy (Shea *et al.*, 1991). Paul and Jeffrey (1985) suggested that *Vibrio proteolytic* has separate adhesive mechanisms for hydrophilic and hydrophobic substrata, and that protein-containing adhesives occur on the latter. Similarly, there is evidence that chemically different adhesives are produced by each type of algal rhizoid. The fouling alga *Ceramium rubrum* produces mucilage with a complex polysaccharide-protein complex from disc-like rhizoids, but a complex polysaccharide only from filamentous rhizoids (Fletcher, 1984b). A further possibility for differences in settlement and attachment in the current study, is the variation in microfouling communities that colonise silicone-coated netting compared with uncoated netting (Hodson & Burke, 1994). Microbial films can significantly change surface energy

(Maki *et al.*, 1994), and chemical cues from these communities can both attract and deter settling macroorgansisms (Wahl, 1989; Clare *et al.*, 1992).

The resistance of the silicone coating to solitary ascidians significantly reduced the total fouling biomass (Figs 57, 65). On this surface ascidians never exceeded 3 cm in length, suggesting that their adhesion strength was not sufficient to support weight and drag forces above this size. This is of considerable benefit in Tasmania because ascidians are often the dominant fouling organism on large-mesh netting (10 cm) used for predator protection. Furthermore, methods to reduce fouling by solitary ascidia are of wide-spread interest as they are major fouling organisms on salmon cages in Norway (Sutterlin & Merrill, 1978), pearl-culture nets in China (Chenxing, 1990), and oyster long-lines in Japan (Arakawa, 1973). Milne (1975b) also reported severe fouling by solitary ascidians in netting evaluation trials in Scotland.

Fouling biomass and mesh occlusion were always lower on the silicone-coated netting than on the uncoated netting. However, the level of fouling that did occur would still necessitate periodic cleaning to maintain cage water exchange. The major disadvantage of current silicone coatings is their lack of abrasion resistance and tear strength (Milne & Callow, 1985), and shore-based handling and cleaning of nets would cause severe damage. However, the greater ease of fouling removal would increase the potential for effective *in situ* cleaning, and relatively light scrubbing could maintain a clean net. Fouling regrowth and recolonisation would also be reduced because the silicone provided a smooth surface that filled the crevices of the netting weave.

The application of a commercial silicone coating (Viridian 2000, International Paints) to fish-cage netting significantly reduced total fouling mass and greatly increased the ease of net cleaning. The flexibility of silicone coatings and the effective reduction of fouling adhesion make them ideal for fish cages that will be cleaned *in situ*, and may provide an effective non-toxic solution to reduce the cost of fouling removal.

CHAPTER 5

Mariculture antifouling:

Polymer carriers for natural and commercial compounds

5.1 Introduction

A promising new area for development of environmentally acceptable antifoulants is the wide range of inhibitory compounds that have been isolated from marine invertebrates, algae and bacteria (Clare, 1996). Many of these compounds are associated with chemical defence against epibiosis and can prevent overgrowth by species that are also rapid colonisers of man-made structures (Wahl, 1989; de Nys *et al.*, 1995). Laboratory bioassays with invertebrate larvae, macroalgal spores and bacteria have shown some compounds to be acutely toxic, but many others to effectively inhibit settlement at non-toxic concentrations (Todd *et al.*, 1993; Holmström & Kjelleberg, 1994; de Nys *et al.*, 1995).

Commercialisation of this technology is still in its infancy, and few field trials are reported in the literature. Those which have been conducted are generally short-term analyses of compounds extracted from marine organisms (eg. Willemsen & Ferrari, 1993), and are mostly aimed at mimicking natural release rates to verify the antifouling role for these compounds (eg. Henrikson & Pawlik, 1995). Field trials and development of analogs of natural compounds are also scarce (eg. Price *et al.*, 1992; Clare *et al.*, 1995). Production of analogs, and elucidation of how their molecular structure affects antifouling activity, is essential for commercialisation because natural compounds are often structurally complex (and so are expensive and difficult to synthesise) and are limited in availability (Targett & Stochaj, 1994). It is noteworthy that many natural antifoulants are only highly active against specific fouling taxa, so broad-spectrum commercial products are likely to contain a suite of compounds each targeted at specific groups (Rittschof *et al.*, 1994; Targett & Stochaj, 1994; de Nys *et al.*, 1995).

This chapter describes field trials of extruded polymers (plastics) that incorporate antifouling compounds. These polymers can be extruded as filaments for fish-cage netting and as rigid mesh for shell-fish containment. Antifouling efficacy was evaluated for polymers incorporating either an algal extract (*Delisea pulchra* or *Laurencia rigida*), an analog of a halogenated furanone (that occurs in *D. pulchra*), or one of four commercial biocides (Busan 11-M1™, Irgarol 1051™, Nopcocide N-96™ and Sea-nine 211™). Antifoulant release from different types of polymer and from polymers with different

antifoulant loadings was also investigated. Analysis of micro- and macro-fouling succession clearly demonstrates the potential for polymer-based delivery of antifoulants, and has identified future directions for research into environmentally acceptable mariculture antifouling.

5.2 Materials and Methods

All field trials were conducted at the Huon Aquaculture Company's lease at Hideaway Bay (Fig. 49, chapter 4).

5.2.1 Polymer-based delivery of antifouling compounds I

The first generation of antifouling polymer was produced from one polymer type (Dupont Elvax® 3165SB, ethylene-vinyl acetate) which incorporated either a commercial biocide (Busan 11-M1™, Irgarol 1051™, Nopcocide N-96™, or Sea-Nine 211™, see Appendix 1) or a crude algal-extract (*Delisea pulchra* or *Laurencia rigida*). Each "antifoulant" was incorporated into the polymer at a loading of 1% (of polymer dry weight), except for the *D. pulchra* extract which was included at 1% and 5%. The polymer was extruded as a 13 cm wide, 400 μm-thick strip.

The treatments were cut into 25 cm strips for field testing and each treatment divided into two groups of an equal number of replicates (or as close as possible where there was odd number of replicates). Each group was attached randomly within a large test frame and immersed at 1 m depth (Fig. 68). Polymer without antifoulant was used as a control. Strips of a commercial silicone product (Viridian 2000) were also included. A total of 72 strips were used, but replication varied between treatments (depending on antifoulant availability, Table 9). The trial commenced on September 17, and fouling development was recorded using close-up and wide-angle underwater photography after 25, 35, 60 and 75 days immersion.

To provide samples for scanning electron microscopy (SEM) 43 smaller strips (3 X 13 cm) were randomly attached to a third frame and fixed underneath one of the larger strip-containing panels (Fig. 68). Six strips of each treatment were included, with the exception of 1% *L. rigida* (2 strips) and 5% *D. pulchra* (5 strips), because of scarcity of material. Two strips (selected randomly) were removed per treatment after 25, 35 and 75 days immersion. During sampling, a central 1 cm by 8 cm block was excised from each strip and prepared for scanning electron microscopy (according to Hodson & Burke, 1994). Each block was cut into eight 1 cm by 1 cm sections for observation.

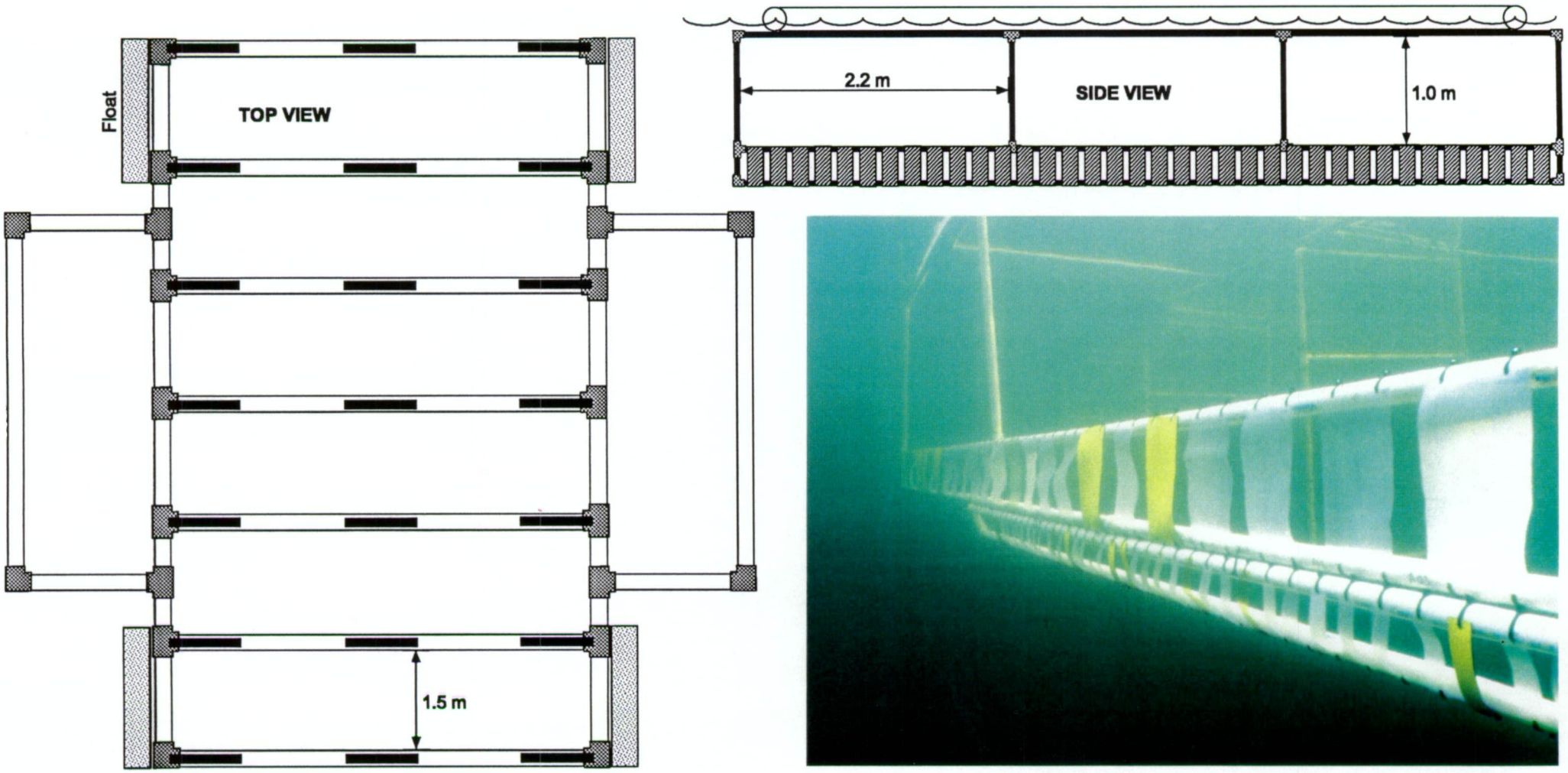


Figure 68. Raft and panel design for trials of antifouling polymers. Two 5 m outriggers (added to the raft used for evaluation of silicone coatings) each held a 6.7 m panel of 36 test strips. The polymers were attached randomly within the 2 panels and were immersed at 1.0 m depth.

Table 9. Number of replicate strips (25 X 13 cm) for the 1st generation of polymer

Antifouling treatment	Number of replicates
Control polymer	7
1% Busan 11-M1	15
1% Irgarol 1051	10
1% Nopcocide N-96	10
1% Sea-Nine 211	9
1% *Delisea pulchra* extract	2
5% *D. pulchra* extract	3
1% *Laurencia rigida* extract	3
Silicone (Viridian 2000)	13

5.2.2 Optimisation of panel design

On December 10 a 4 m long frame (Fig. 69) was immersed to quantify the effect of panel shape on recruitment and distribution of fouling species. This was conducted to refine field trials with future antifouling-polymer strips. Four panel shapes were tested: two types of cylindrical panel (22 cm and 15 cm circumference) and two types of flat rectangular panel (22 cm and 15 cm wide). Panels were constructed from new polymer sheets (Dupont Elvax® 3165SB) without antifoulant added, and four replicates were included for each shape. The replicates were positioned in a block design with each of the 4 treatments distributed randomly within the 4 sections of the frame.

After 60 days immersion (February 9) the panels were detached, transferred to containers with 10% formalin in seawater, and refrigerated (in the dark) until processing.

The attachment point and wet weight for each species was recorded from each panel. Wet weight was recorded after blotting samples dry and weighing to ±0.0001 g. The distribution of each species within each panel was then quantified in terms of total mass and the number of individuals occurring at the panel edge and centre. The edge of the rectangular panels was defined as a zone around the periphery which was of equal surface area to the region it encompassed (the centre). The edge of the cylinders was the upper and lower quarters combined, and the centre was the region in between. Each side (East and West) of the flat panels was analysed separately.

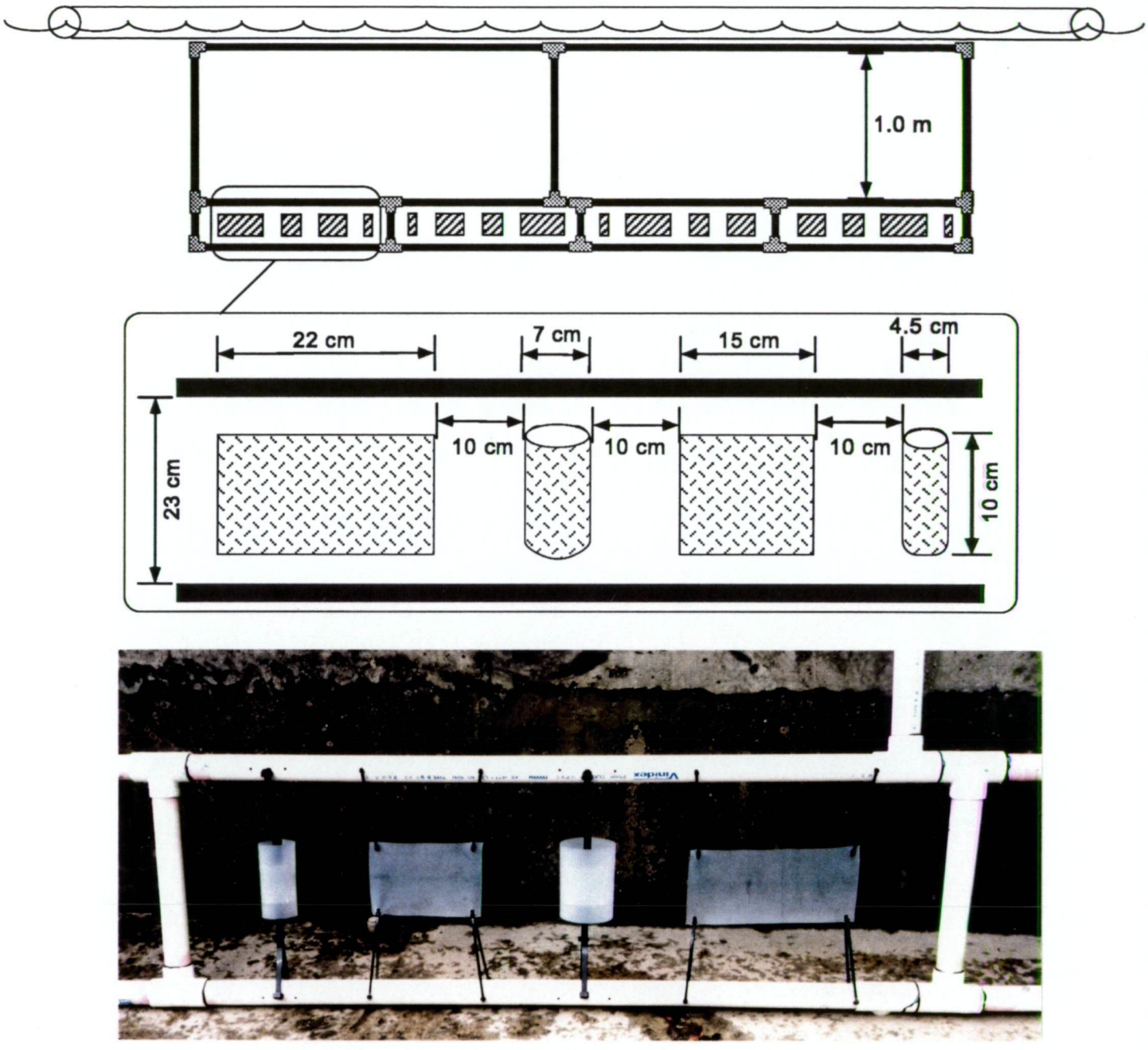


Figure 69. Frame and panel design for quantifying the effect of panel shape and size on fouling development. The large and small cylinders had a circumference of 22 cm and 15 cm, respectively. Four replicates were included for each shape. One replicate of each shape was placed randomly within each of the 4 sections of the frame.

5.2.3 Polymer-based delivery of antifouling compounds II

In the second polymer-antifoulant evaluation, 8 types of polymer (Table 10) were combined with either Sea-nine 211 or an analog of a halogenated furanone isolated from *D. pulchra* (see de Nys *et al.*, 1995). The polymers were chosen to give a range of release rates. A polymer equivalent to that used in trial 1 (Dupont Elvax® 470) was used, but with antifoulants at 1%, 5% and 10% loading. Twenty-five treatments were used in total: 8 controls (each polymer without antifoulant), 10 combinations of Sea-nine 211 and 7 combinations of the furanone (Table 11).

Polymers were cut into 18.5 cm-long strips and attached to sections of PVC piping (Fig. 70). The panels (n = 248) were attached to 6 rows of a large polyethylene raft (Fig. 71). Rows 1 and 2 were used for release rate measurement, rows 3, 4 and 5 for photographic records of fouling development and row 6 (10 panels only) for SEM samples. The experiment was set up as a 1-way block design in rows 1 to 5, with randomised placement of duplicates for each treatment in each row. However, not all treatments were fully replicated because of limited quantity of some polymer types and of the furanone (Table 11).

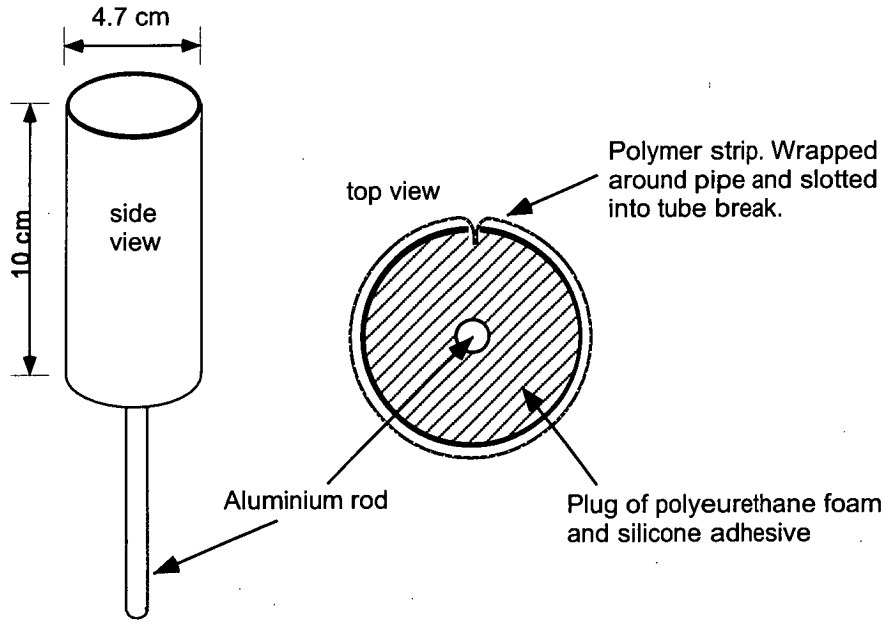


Figure 70. Panel design for presentation of antifouling polymers (trial 2). The PVC tubes were cut down one side, and the gap forced open to insert the ends of each polymer strip. The strips and an alumium support rod were glued into place usign polyeurethane boating foam and marine silicone adhesive.

Table 10. Polymer types evaluated in trial II.

Company	Polymer name/code	Polymer type
Dupont	Elvax® 470[a]	Ethylene-vinyl acetate copolymer (EVA)[b]
elf atochem	Evatane® 1005 VN5	Ethylene-vinyl acetate copolymer (EVA)[b]
elf atochem	Evatane® 1020 VN3	Ethylene-vinyl acetate copolymer (EVA)[c]
elf atochem	Evatane® 28.03	Ethylene-vinyl acetate copolymer (EVA)[d]
Kemcor	HD 6095	High-density polyethylene (HDPE)
Shell	HET 6100	Polypropylene (PP)
Dupont	Surlyn® 1707	Na^+ ionomer
BASF	Lucalen® A	Copolymer of ethylene and acrylic acid

[a] equivalent to Elvax™ 3165 SB, used in trial 1
[b] 8% vinyl acetate
[c] 18% vinyl acetate
[d] 28% vinyl acetate

Table 11. Polymer and antifoulant combinations evaluated in trial II. Ten replicates were used for each treatment, except where the number of replicates is specified in brackets.

Polymer type	Percent antifoulant furanone[a]	Sea-nine 211	Halogenated
Dupont Elvax® 470	1%	✓	✓
Dupont Elvax® 470	5%	✓	✓ (9)
Dupont Elvax® 470	10%	✓	✓
elf atochem Evatane® 1005 VN5	1%	✓ (6)	×[b]
elf atochem Evatane® 1020 VN3	1%	✓ (7)	×
elf atochem Evatane® 28.03	1%	✓	×
Kemcor HD 6095	1%	✓	✓
Shell HET 6100	1%	✓	✓ (5)
Dupont Surlyn® 1707	1%	✓	✓
BASF Lucalen® A	1%	✓	✓

[a]The halogenated furanone was not included in all polymers because of limited quantity
[b] × = combination not tested

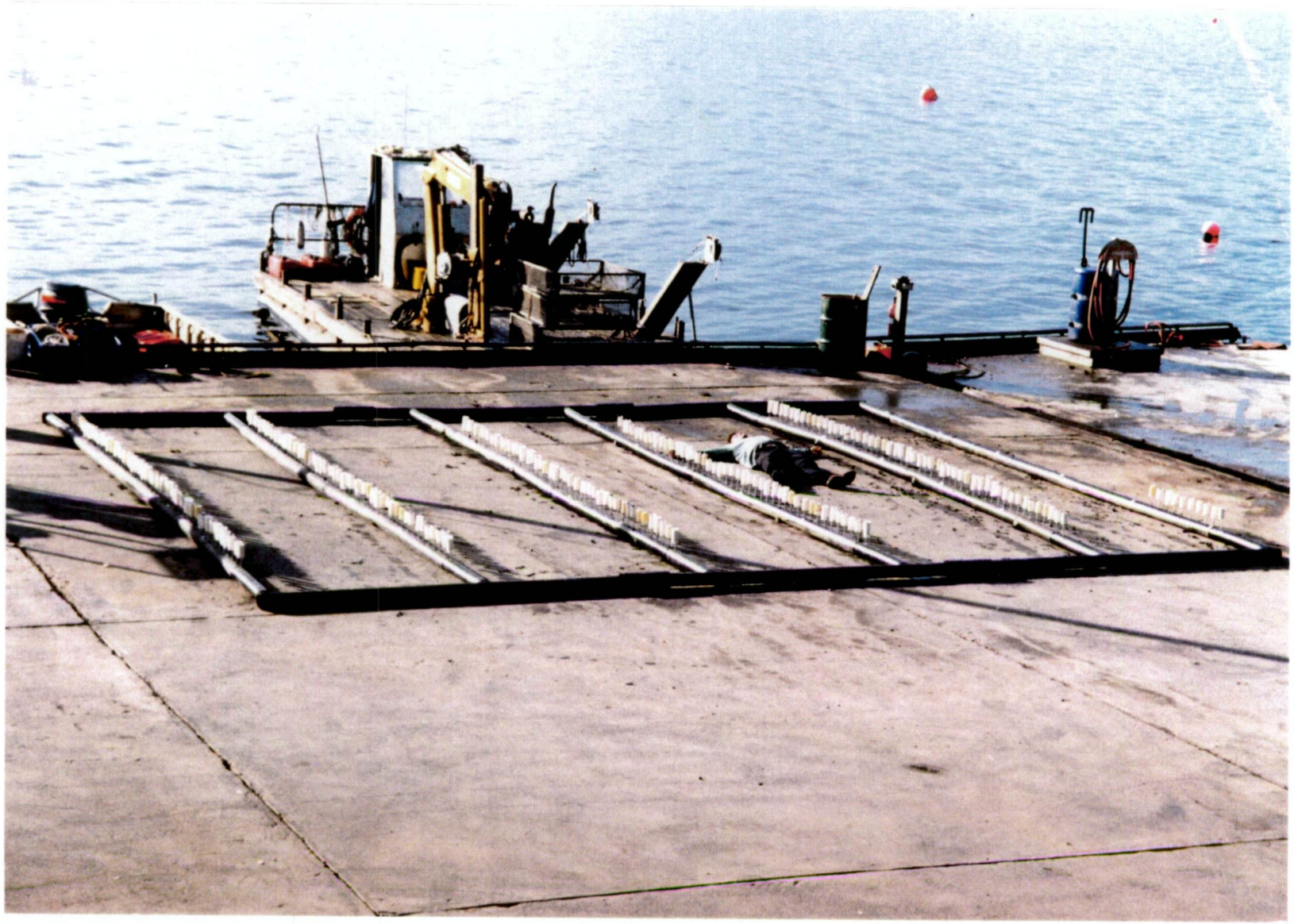


Figure 71. Raft and panel design for evaluation of antifouling polymers (trial 2). Polymer strips were wrapped around 10 cm-high PVC tubes (4.7 cm outer diameter) that were fixed 8 cm above the raft. Eight polymer types were evaluated, and antifoulants were included at 3 concentrations in polymer A. Duplicates of each treatment and control were placed randomly within each of the first 5 rows on the raft. Rows 1 and 2 were used for analysis of antifoulant release rates. Rows 3, 4 and 5 were used for photographic records of fouling development. Row 6 polymers (10 samples only) were used for scanning electron microscopy.

The 10 panels in row 6 were included to allow SEM observation of colonisation patterns that may have been observed in rows 1 to 5. The panels were made from any excess material not needed in rows 1-5, and included 2 panels of Sea-nine 211 in Elvax (1%), 2 panels of Sea-nine 211 in Elvax (5%), 3 panels of Sea-nine 211 in Elvax (10%), 1 panel of the furanone in Elvax (1%), 1 panel of the furanone in Elvax (10%) and 1 panel of the furanone in Surlyn.

On May 6, 1996, the raft was immersed at 1 m depth. After 20 days immersion 1 cm by 3 cm samples were cut from each polymer-antifoulant panel in rows 1 and 2, and close-up underwater photographs were taken of all panels in rows 3, 4 and 5. Sampling was repeated at 2 to 3 week intervals (in most cases) for 6 months (Table 12). Because of severe fouling on most panels, wide-angle photography was used after 125 days. Sampling of rows 1 and 2 was reduced after 47 days because most treatments failed to inhibit fouling. Samples cut from rows 1 and 2 were sent to UNSW for quantification of remaining antifoulant (via gas chromatography-mass spectrometry).

Table 12. Sampling schedule for polymer antifoulants evaluated in trial II.

Date	Days immersed	Release rate samples	Close-up photography	Wide-angle photography
May 26	20	all treatments	✓	
Jun 9	34	all treatments	✓	
Jun 22	47	Sea-nine 211 in Elvax® 470[a]	✓	
Jul 6	61	Sea-nine 211 in Elvax® 470[a]	✓	
Aug 4	90	Sea-nine 211 in Elvax® 470[a]	✓	
Aug 24	110	Sea-nine 211 in Elvax® 470[a]	✓	
Sep 8	125	Sea-nine 211 in Elvax® 470[a]		✓
Sep 29	146	Sea-nine 211 in Elvax® 470[a]		✓
Oct 13	160	Sea-nine 211 in Elvax® 470[a]		✓
Nov 3	181	Sea-nine 211 in Elvax® 470[a]		✓
Dec 1	209	Sea-nine 211 in Elvax® 470[a]		✓

[a] 1%, 5% and 10% loadings

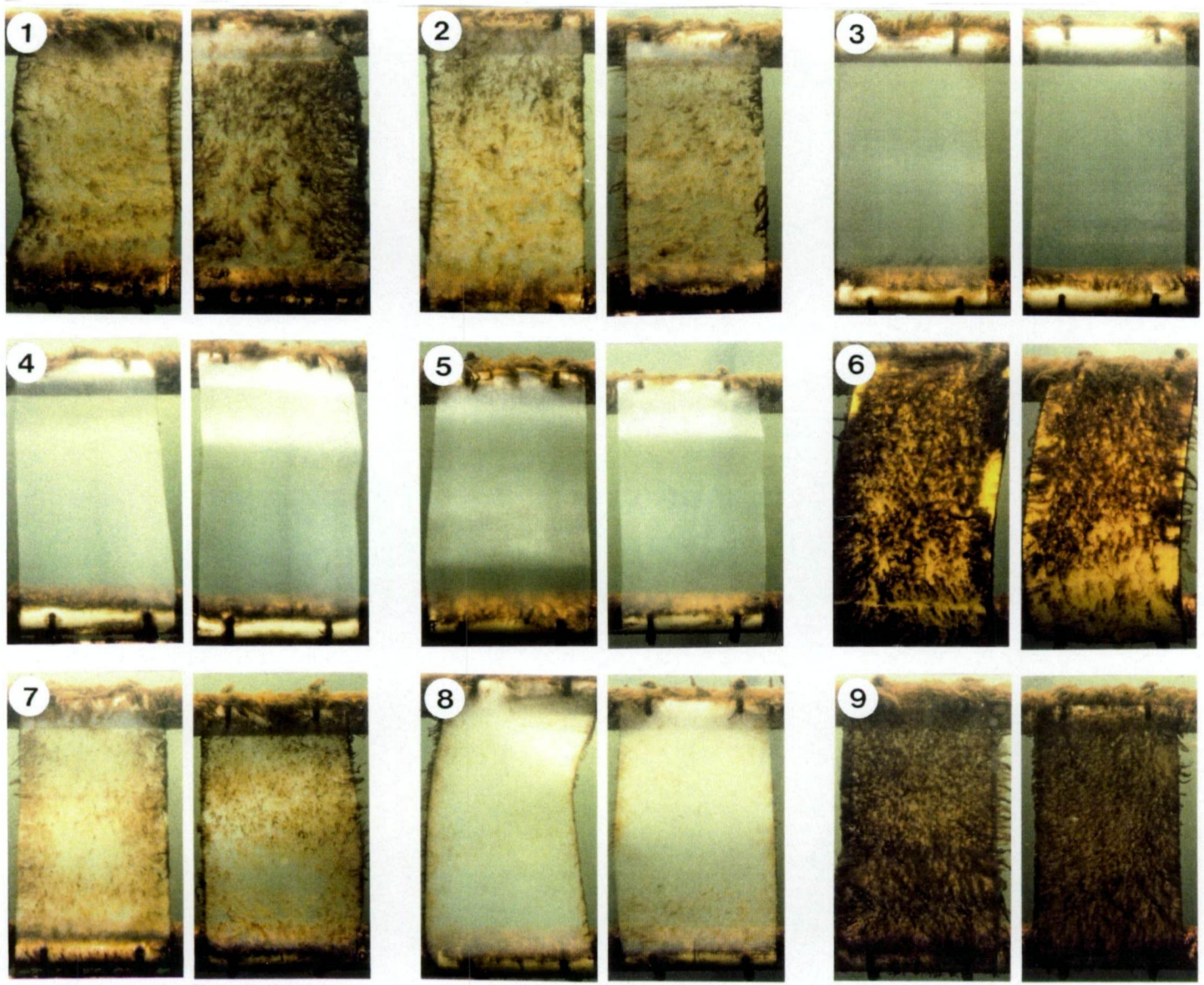
5.3 Results

5.3.1 Polymer-based delivery of antifouling compounds I

The effectiveness of both the commercial and natural compounds to prevent fouling was demonstrated after 25 days immersion (Fig. 72). Underwater photographs showed that polymer with Irgarol 1051, Nopcocide N-96 or Sea-nine 211 was unfouled, and polymer with 5% *D. pulchra* extract had limited fouling. However, the control polymer, polymers containing 1% Busan 11-M1, 1% *D. pulchra* extract or 1% *L. rigida* extract, and the silicone coating were heavily fouled. Furthermore, the polymer with *L. rigida* extract had far greater fouling than the control polymer. The dominant fouling at this time was a tube-dwelling diatom, a common organism on salmon cages at this time of year (Hodson & Burke, 1994). Of the four effective antifouling treatments, Irgarol 1051 and Sea-nine 211 proved superior and still performed well after 60 days immersion (Fig. 73). The polymer with 5% *D. pulchra* extract was fouled after 35 days, and the polymer with Nopcocide N-96 after 60 days (Fig. 73). The fouling sequence on Irgarol 1051 was noticeably different from other polymers; the polymer was colonised at the edges by hydroids, which eventually covered the panel surface and were then heavily colonised by epiphytic algae (Fig. 74). Algal growth primarily occurred on the hydroids and was severely restricted on the polymer surface.

Polymer observation with SEM clearly demonstrated the same relative performance of the four most effective treatments, but gave an earlier indication of their failure. Polymer with Sea-nine 211 performed best, and was almost completely free of fouling after 25 days immersion (Fig. 75). The only fouling at this time was from small colonies of bacteria (Fig. 75C). In comparison, the other two commercial antifoulants displayed a greater level and diversity of colonisation after 25 days. Polymer containing Irgarol 1051 was colonised by high numbers of bacteria (Fig. 76) and a variety of stalked protozoa. Polymer with Nopcocide N-96 was extensively colonised by dense bacterial colonies and associated mucilage, and by a variety of diatom species (Fig. 77). Polymer containing 5% *D. pulchra* extract was extensively colonised by diatoms within a thick mucilaginous layer (Fig. 78) and large diatoms such as stalked *Licmophora* and invertebrates were common on the surface.

After 35 days immersion the polymer with Sea-nine 211 was extensively colonised by diatoms, protista and bacteria (Fig. 79), but fouling was less severe than on Irgarol 1051 and Nopcocide N-96. Most of the Irgarol 1051 samples were colonised by the hydroid *Obelia australis* (Fig. 80A), and Nopcocide N-96 was extensively fouled by



1) Control polymer
2) 1% Busan 11-M1
3) 1% Irgarol 1051
4) 1% Nopcocide N-96
5) 1% Sea-nine 211
6) Silicone coating
7) 1% *D. pulchra* extract
8) 5% *D. pulchra* extract
9) 1% *L. rigida* extract

Figure 72. Fouling growth on antifouling polymers after 25 days immersion. The dominant fouling organism was a tube-dwelling diatom.

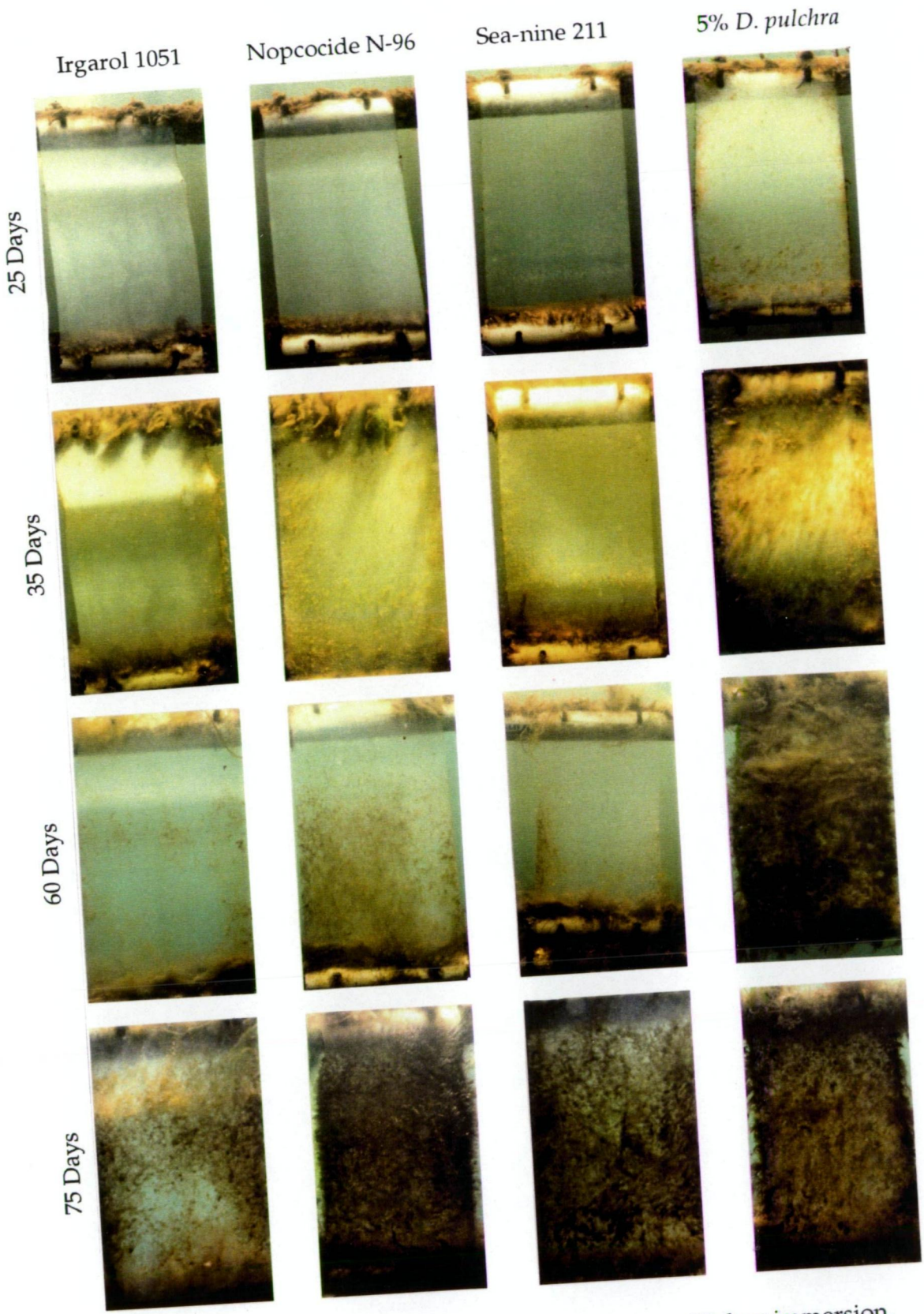


Figure 73. Performance of antifouling polymers over 75 days immersion.

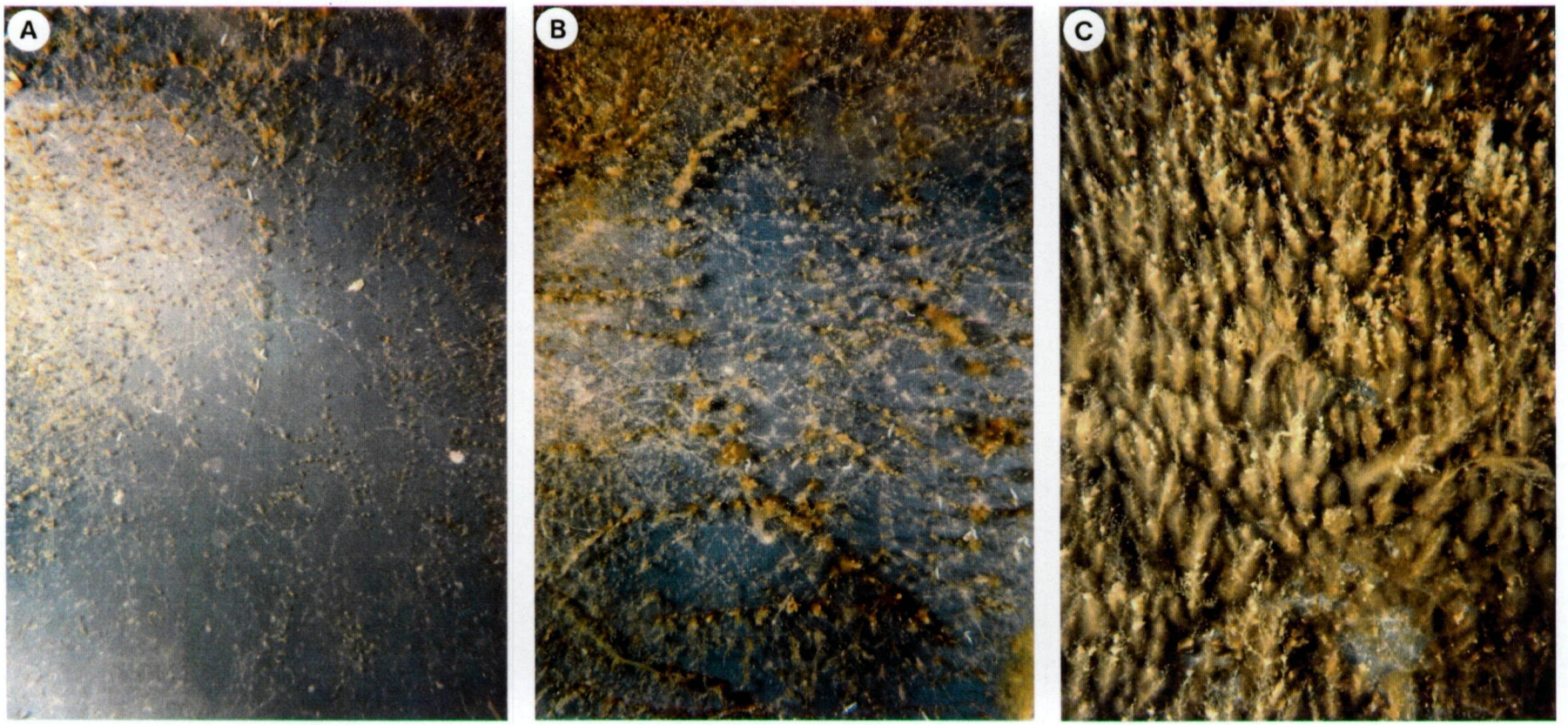


Figure 74. Fouling sequence on polymer containing 1% Irgarol 1051. The hydroid *Obelia australis* was the primary macrofouling organism, and extensively covered the panels. Epiphytic diatoms heavily colonised the hydroids, but were severely restricted on the polymer surface. A = 35 days, B = 60 days, C = 75 days.

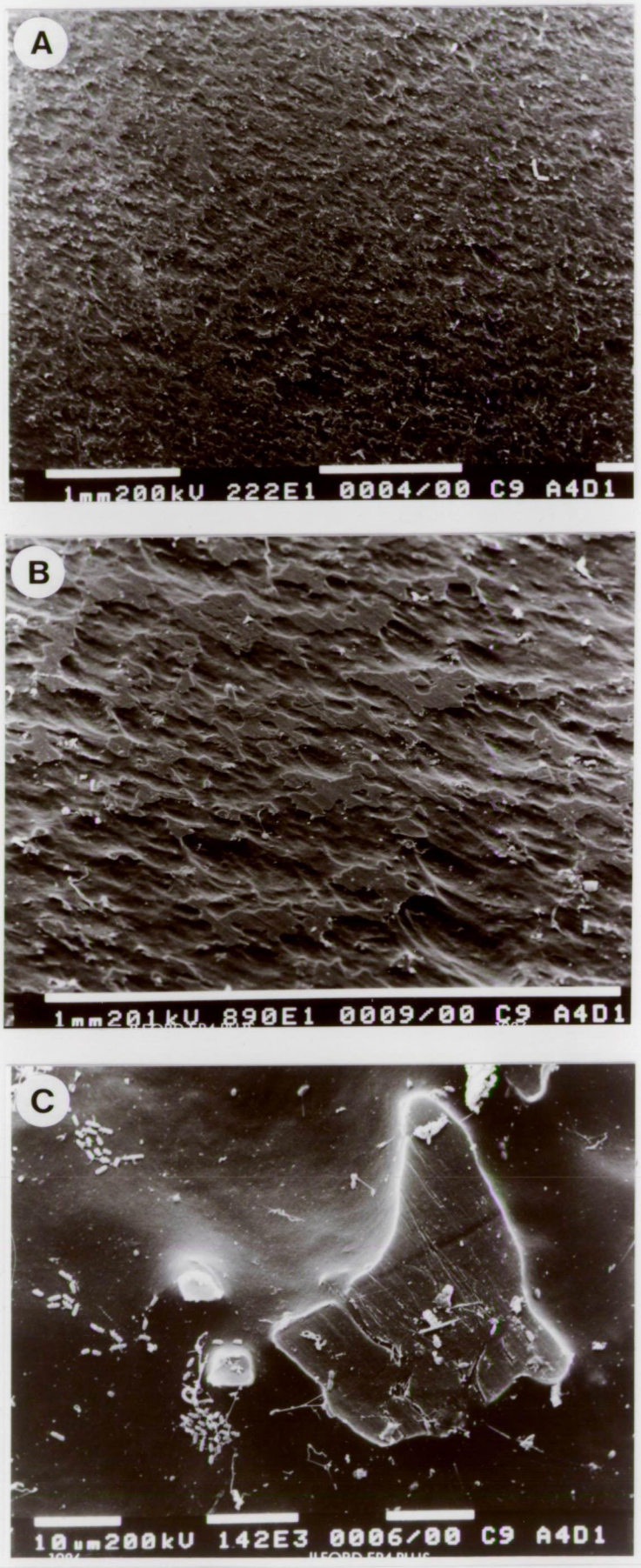


Figure 75. Polymer with Sea-nine 211 after 25 days immersion. Small bacterial colonies (C) were the only fouling present.

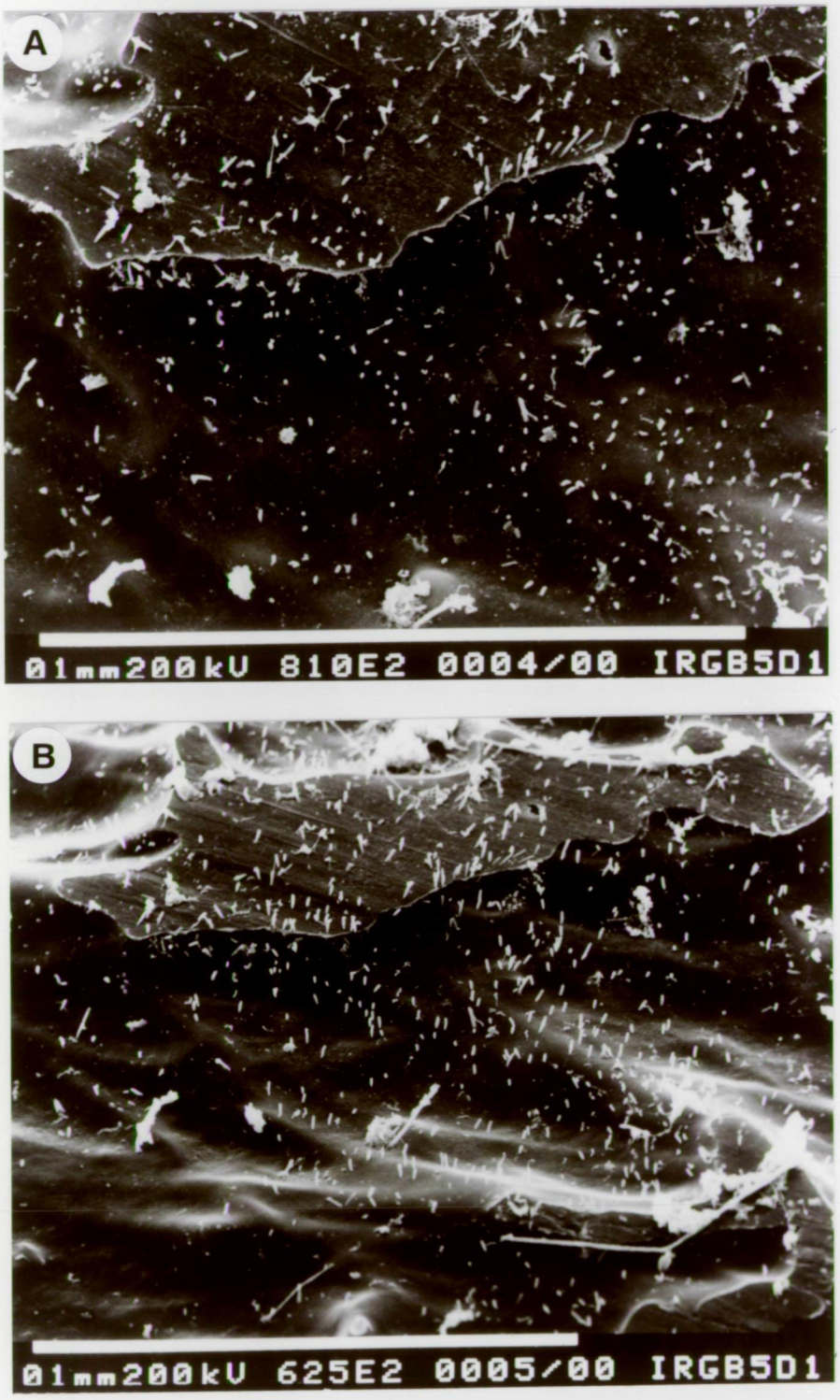


Figure 76. Polymer with Irgarol 1051 after 25 days immersion. High numbers of bacteria had colonised the surface.

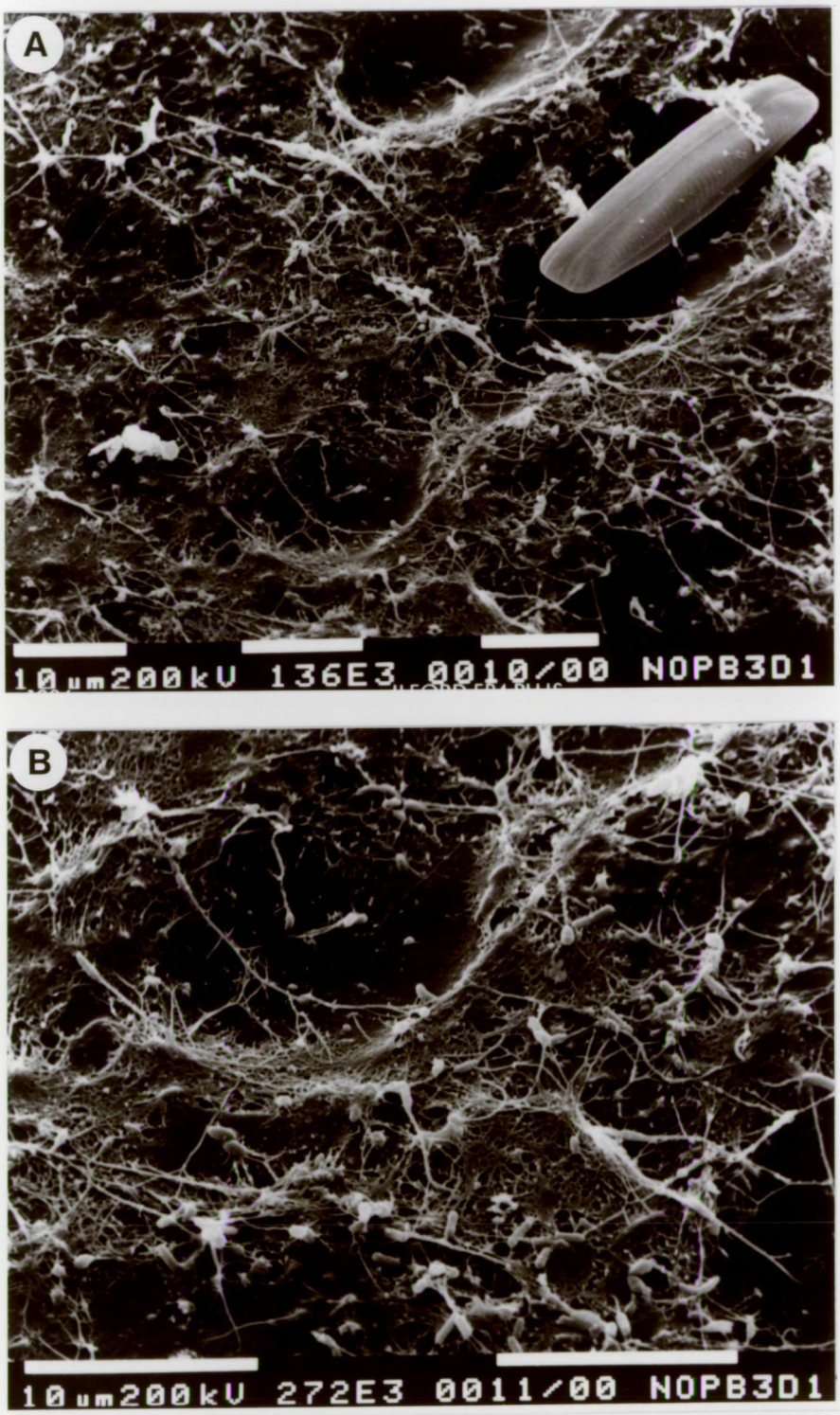


Figure 77. Polymer with Nopcocide N-96 after 25 days immersion. Most of the surface was colonised by dense bacterial colonies within a mucilaginous layer. Diatoms (A) were also common on the surface.

Figure 78. Polymer with 5% *Delisea pulchra* extract after 25 days immersion. The polymer was extensively covered by diatoms within a thick mucilaginous layer. Invertebrates grazing on the surface were common (B).

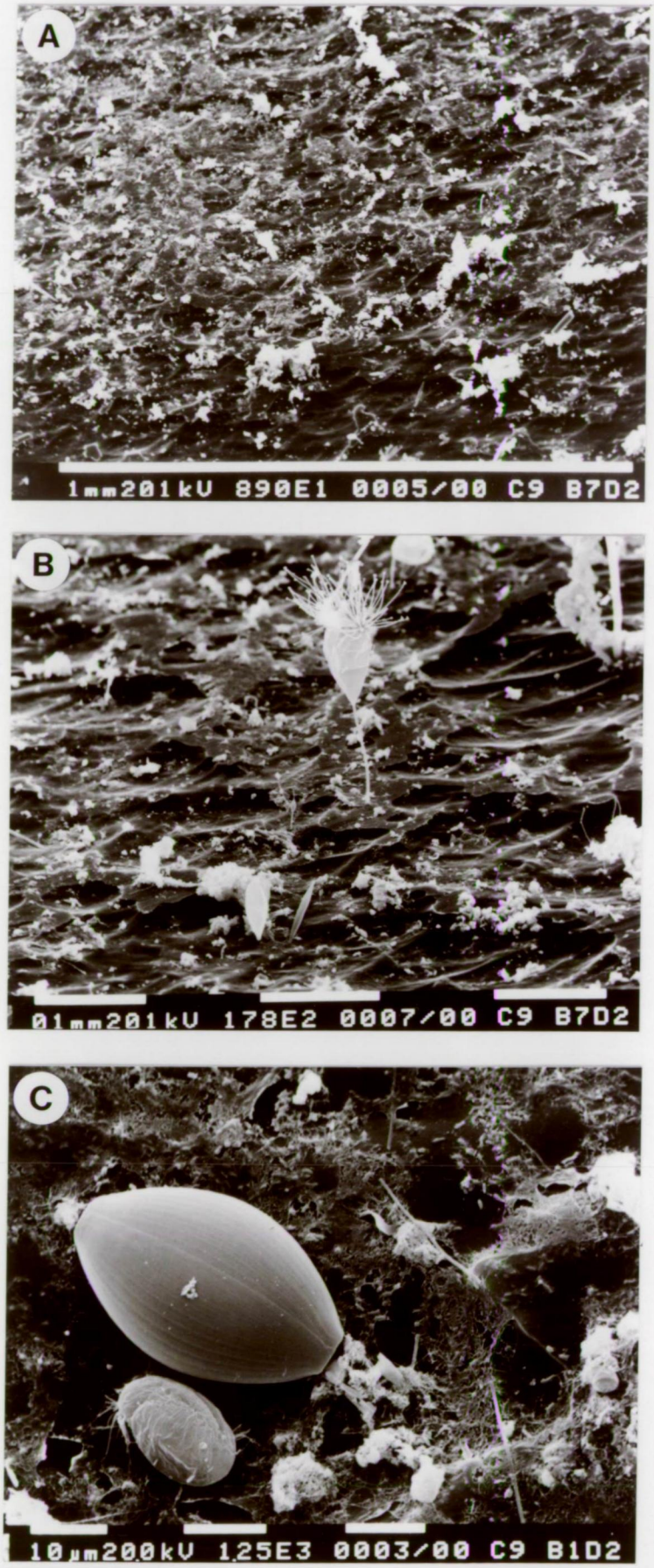


Figure 79. Polymer with Sea-nine 211 after 35 days immersion. The surface was colonised by a diverse range of microorganisms, including stalked Suctoria (B), ciliates and diatoms (C).

Figure 80. Fouling development after 35 days immersion on polymers with Irgarol 1051 (A) and Nopcocide N-96 (B). Irgarol 1051 was colonised by a diversity of microorganisms and the hydroid *Obelia australis*. Large diatom colonies, such as *Licmophora* were common on Nopcocide N-96.

microorganisms and supported large colonies of *Licmophora* (Fig. 80B). After 60 days a diverse microfouling community covered Sea-nine 211 and Irgarol 1051, and hydroids were abundant on the latter treatment.

5.3.2 Optimisation of panel design

The shape of the polymer test panels significantly affected fouling biomass (Fig. 81), and greater fouling developed on the smaller cylinders (per cm^2) than on either of the flat plates. The results also indicated that flat panels were subject to an orientation effect, with lower (but not significantly different) biomass on the western sides. The western sides faced toward the raft and net panels (for the silicone trial), and may have received some shading.

Biomass and count comparisons excluded those organisms attached to the 400 µm-thick edge of the panels. However, the biomass on these edges was often significant, and on the flat plates it was similar to that of the primary surface. For example, the mean biomass on the primary surface of the small plates was 1.53 ± 0.12 g (s.e.) and on the 400 µm-thick edges it was 1.42 ± 0.34 g (s.e.). The fouling distribution across the flat surfaces clearly indicated an edge effect, with significantly greater biomass on the edges than the centres (Table 13). In comparison, fouling was more evenly distributed across the cylinders, although there was some trend toward greater fouling at the edges (Table 13).

The panels were fouled almost exclusively by five species of macroalgae, with *Polysiphonia infestans* abundant on each replicate (Tables 14, 15). The small cylinders had twice as many *P. infestans* plants as the large cylinders, and nearly four times as many as the flat panels. *Lomentaria australis* was more common on the cylinders than the flat plates, and *Brongniartella australis* was more common on the small cylinder than other shapes. These latter two species were rare or absent from the centres of the flat panels, but common in the centres of the cylinders (Fig. 82).

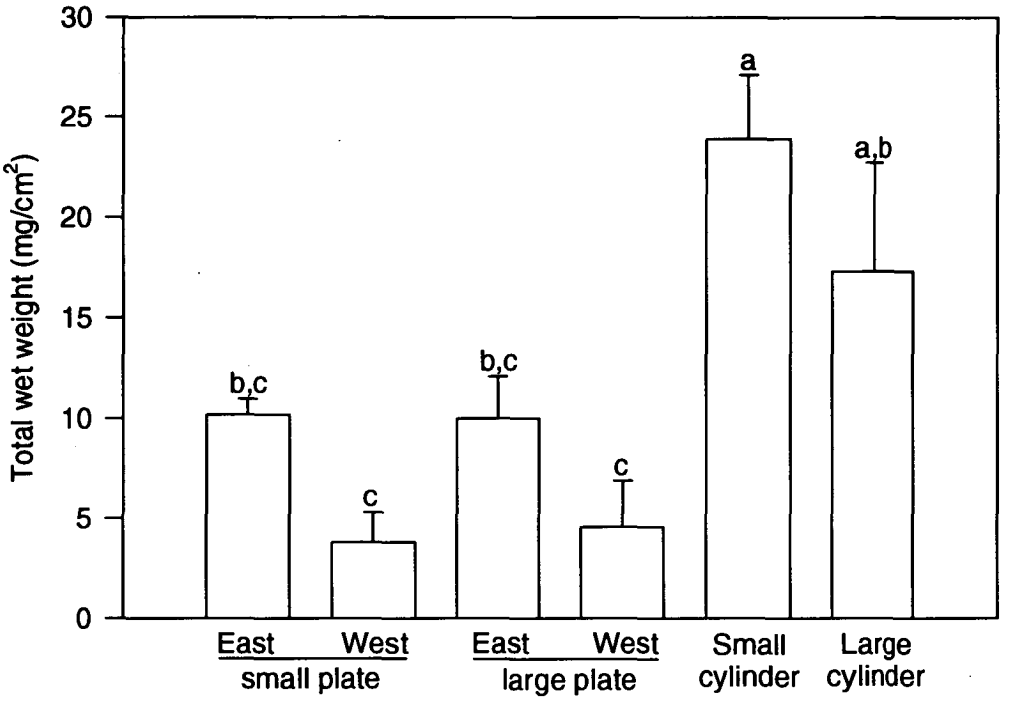


Figure 81. Total fouling mass (cm^{-2}) on each of the four types of panel. The eastern and western faces are plotted separately for the flat plates. Columns are the mean of 4 samples, except for the small cylinder (n=3). Bars = standard error. Columns sharing the same letter are not significantly different ($P > 0.05$, Tukey's test)

Table 13. Distribution of algal biomass on 4 types of test panel. Figures are mean wet weight (grams)/100cm^2 ± standard error

Area	Small cylinder	Large cylinder	Small plate[a]	Large plate[a]
Centre	0.93 ± 0.12	0.57 ± 0.22	0.28 ± 0.07	0.24 ± 0.11
Edge	1.46 ± 0.27	1.16 ± 0.32	0.74 ± 0.09*	0.74 ± 0.12*
centre:edge ratio	1 : 1.6	1 : 2.0	1 : 2.7	1 : 3.1

[a] Eastern face

* edge significantly greater than centre ($P < 0.05$, t-test)

Table 14. Biomass of algal species on 4 types of test panel. Figures are mean wet weight (grams)/100cm^2 ± standard error

Species	Small cylinder	Large cylinder	Small plate[a]	Large plate[a]
Brongniartella australis	0.16 ± 0.07	0.10 ± 0.06	0.07 ± 0.03	0.03 ± 0.02
Ceramium tasmanicum	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.02
Lomentaria australis	0.35 ± 0.06	0.40 ± 0.22	0.04 ± 0.02	0.03 ± 0.02
Polycerea nigrescens	0.02 ± 0.01	0.01 ± 0.01	0.07 ± 0.02	0.06 ± 0.02
Polysiphonia infestans	1.78 ± 0.29	1.18 ± 0.32	0.72 ± 0.04	0.77 ± 0.10

[a] Eastern face

Table 15. Counts of individual plants on 4 types of test panel. Figures are mean number of plants/100cm^2 ± standard error

Species	Small cylinder	Large cylinder	Small plate[a]	Large plate[a]
Brongniartella australis	3.3 ± 0.4	1.2 ± 0.4	1.3 ± 0.5	1.0 ± 0.4
Ceramium tasmanicum	2.2 ± 0.6	0.9 ± 0.6	0.5 ± 0.3	0.6 ± 0.3
Lomentaria australis	3.3 ± 1.3	3.0 ± 0.9	1.3 ± 0.5	1.1 ± 0.5
Polycerea nigrescens	2.7 ± 0.8	1.7 ± 1.2	7.5 ± 2.5	7.2 ± 2.1
Polysiphonia infestans	56.8 ± 2.1	24.9 ± 2.7	14.5 ± 1.3	16.1 ± 1.4

[a] Eastern face

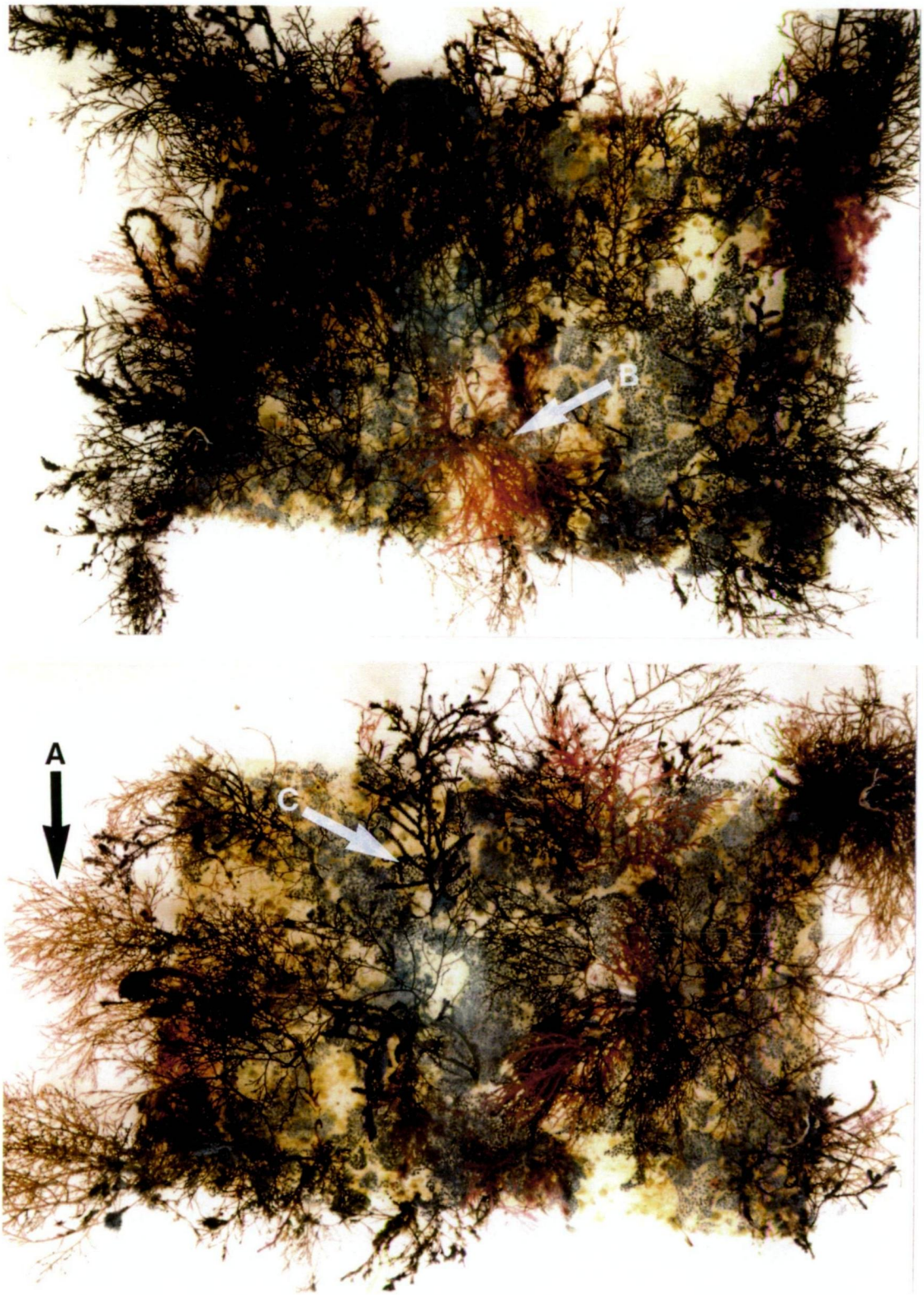


Figure 82. Polymer strips from 2 small cylindrical test panels. Cylindrical panels developed significantly more fouling than flat panels, and fouling was more evenly distributed over their surface. A = *Polysiphonia infestans*, B = *Lomentaria australis*, C = *Brongniartella australis*.

5.3.3 Polymer-based delivery of antifouling compounds II

After 20 days immersion diatom growth covered all control polymers (Fig. 83), but the furanone and Sea-nine 211 effectively prevented fouling (Figs 84-85, Table 16). The furanone performed well in Elvax and Lucalen, but was ineffective in the Kemcor HDPE, Shell PP, and Surlyn. Sea-nine 211 prevented fouling on all polymer types except the Shell PP and Surlyn.

After 47 days immersion diatom growth had increased on all control polymers (Fig. 86), and the antifouling performance was greatly reduced for polymers containing the furanone (Fig. 87). The severity of fouling on the controls varied with polymer type, and Kemcor HDPE, Shell PP, and Surlyn supported greater fouling growth than Lucalen and the 4 types of EVA (Fig. 86). Fouling of polymers containing the furanone was similar to the controls (Fig. 87), except for Elvax (10% loading) and Kemcor HDPE which limited fouling growth. The 10% loading was fouled only by tube-dwelling amphipods (Fig. 88) and a thin diatom film, rather than the large tufts of diatoms on the other panels. The tube-dwelling diatom was occasionally found on other panels, and was common on the Elvax 5% loading. However, amphipod tubes were abundant on all replicates of the 10% loading. These tubes were typically surrounded by clear zones where the amphipods had grazed on the surrounding diatoms. Sea-nine 211 still prevented fouling growth on Elvax (5% and 10% loadings), but the other panels were fouled by diatoms (Fig. 89). Shell PP and Surlyn supported tufts of diatoms, but all other fouled panels were colonised only by a diatom film (Table 16). The film was poorly adhered to most polymers and sections had become detached from many replicates.

Fouling growth developed rapidly on the controls and furanone polymers after 47 days immersion, and by 110 days all were heavily fouled by macroalgae (Figs. 90-91, Table 16). *Ulva* had preferentially settled on some types of control polymer, and there were more plants on Lucalen than any other polymer (Fig. 90). After 110 days Sea-nine 211 still prevented fouling on Elvax (5% and 10% loading), and fouling had not progressed beyond a diatom film except on Shell PP and Surlyn (Fig. 92, Table 16). Sea-nine 211 remained effective in Kemcor HDPE, Lucalen and the 4 types of EVA throughout the trial, whilst the controls developed heavy fouling (eg. Fig. 93). Even after 209 days immersion polymers with Sea-nine 211 were fouled only by diatoms (Fig. 94). It is noteworthy that severe fouling occurred on all structures adjacent to the polymers, and in fact the raft had to be cleaned every 2-3 weeks so that the polymers could be photographed.

Analysis of the quantity of antifoulant remaining in each polymer during the trial demonstrated varying release rates for each polymer-antifoulant combination (Figs 95-96). Analysis of polymer samples prior to immersion (day 0) also showed that the initial loadings were inaccurate and the starting concentration varied greatly. In particular, the treatments for the furanone were markedly less than the corresponding Sea-nine 211 treatments. A common trend for all polymer types and concentrations was a high initial release rate during the first 20 days, and then a gradual decline in release. Elvax maintained a relatively constant release rate for Sea-nine 211 after 20 days, and demonstrated that only small concentrations of this compound were required for effective antifouling (Fig. 96).

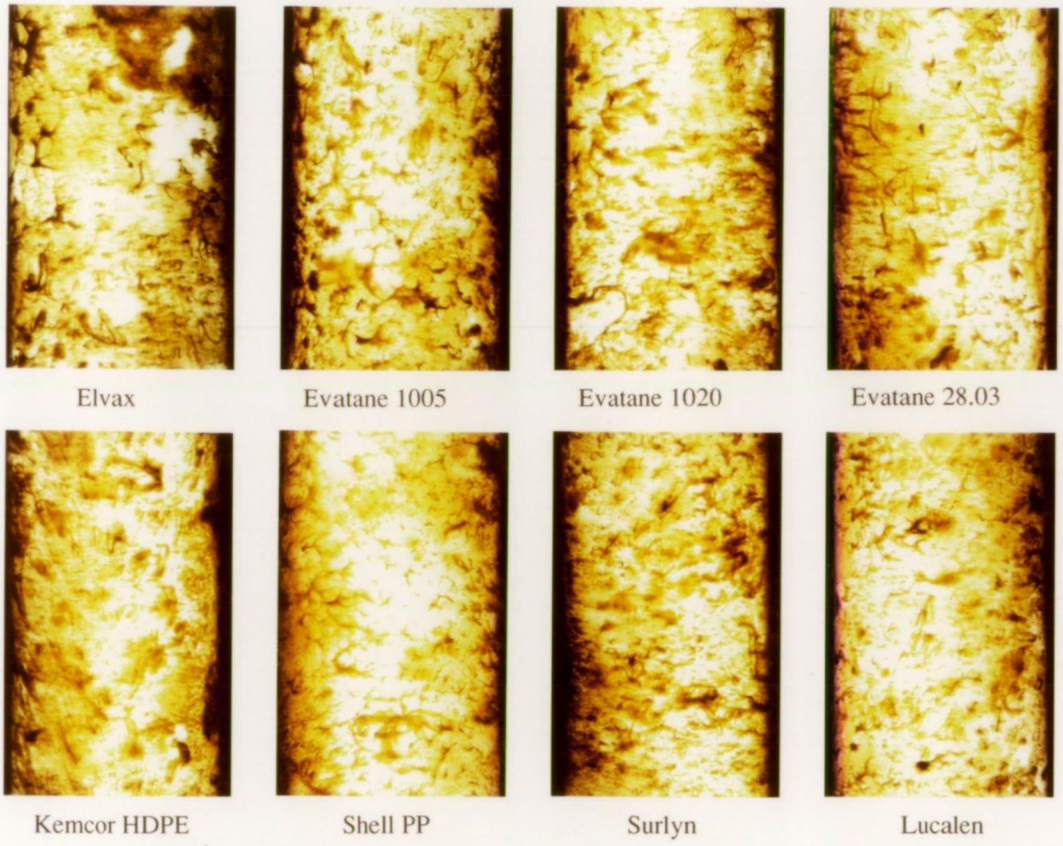


Figure 83. Control polymers after 20 days immersion.

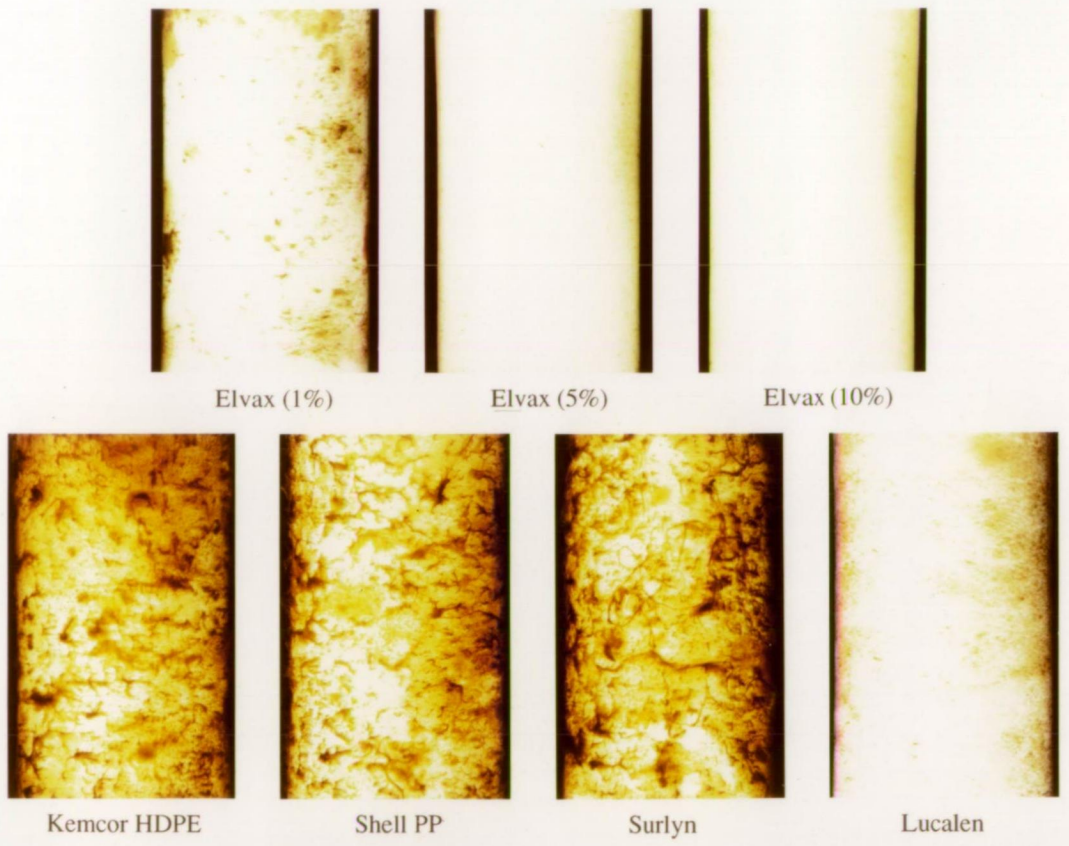


Figure 84. Polymers with the halogenated furanone after 20 days immersion.

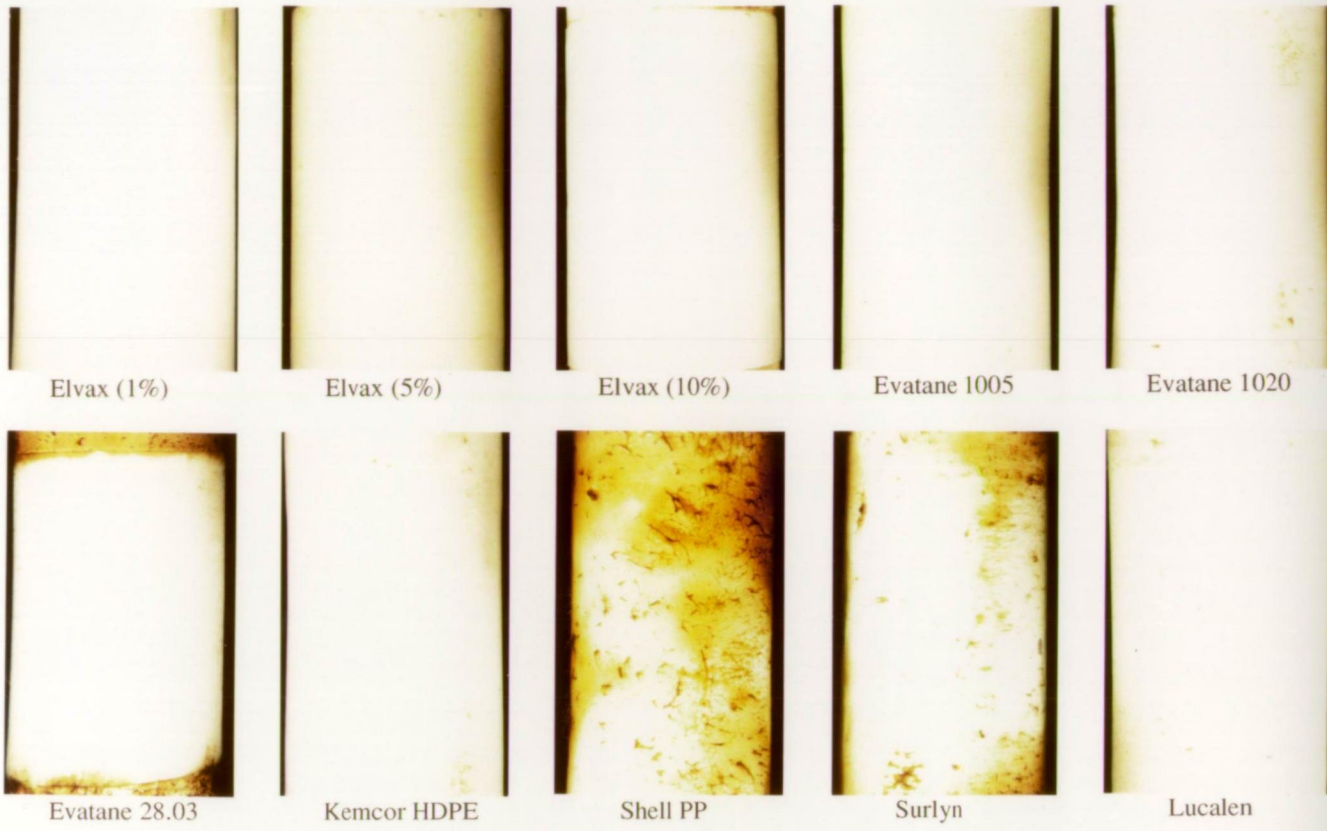


Figure 85. Polymers with Sea-nine 211 after 20 days immersion.

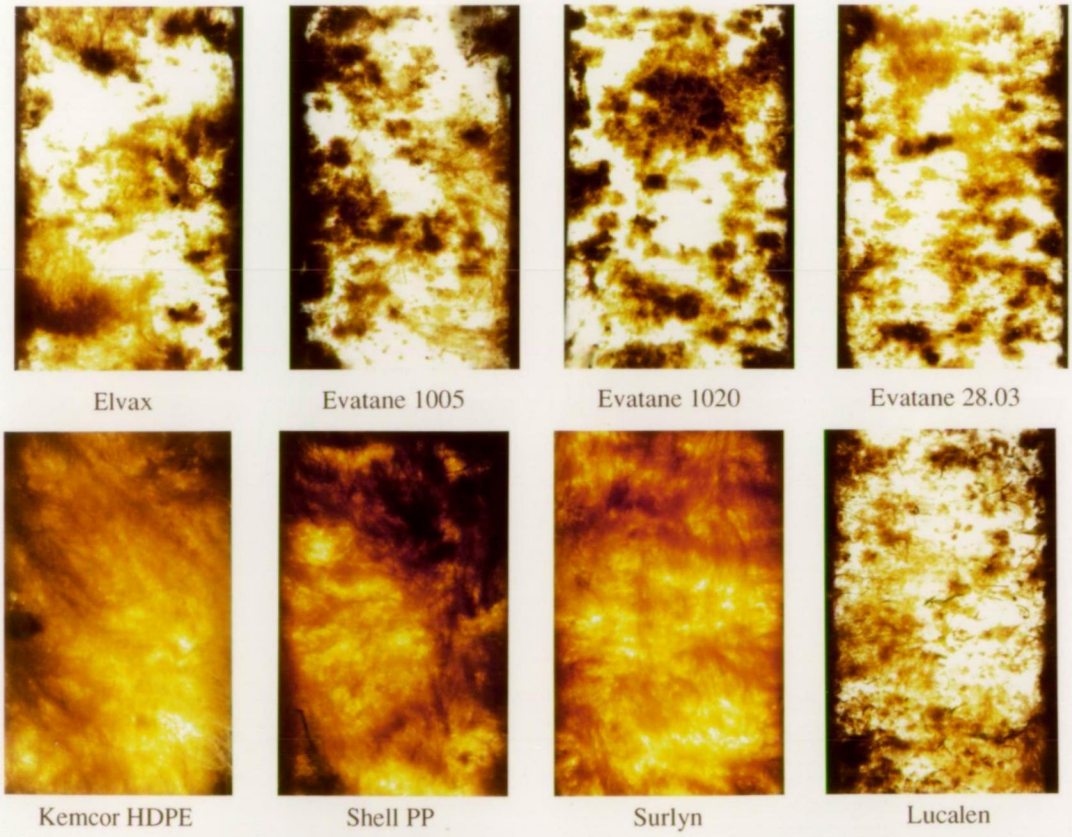


Figure 86. Control polymers after 47 days immersion.

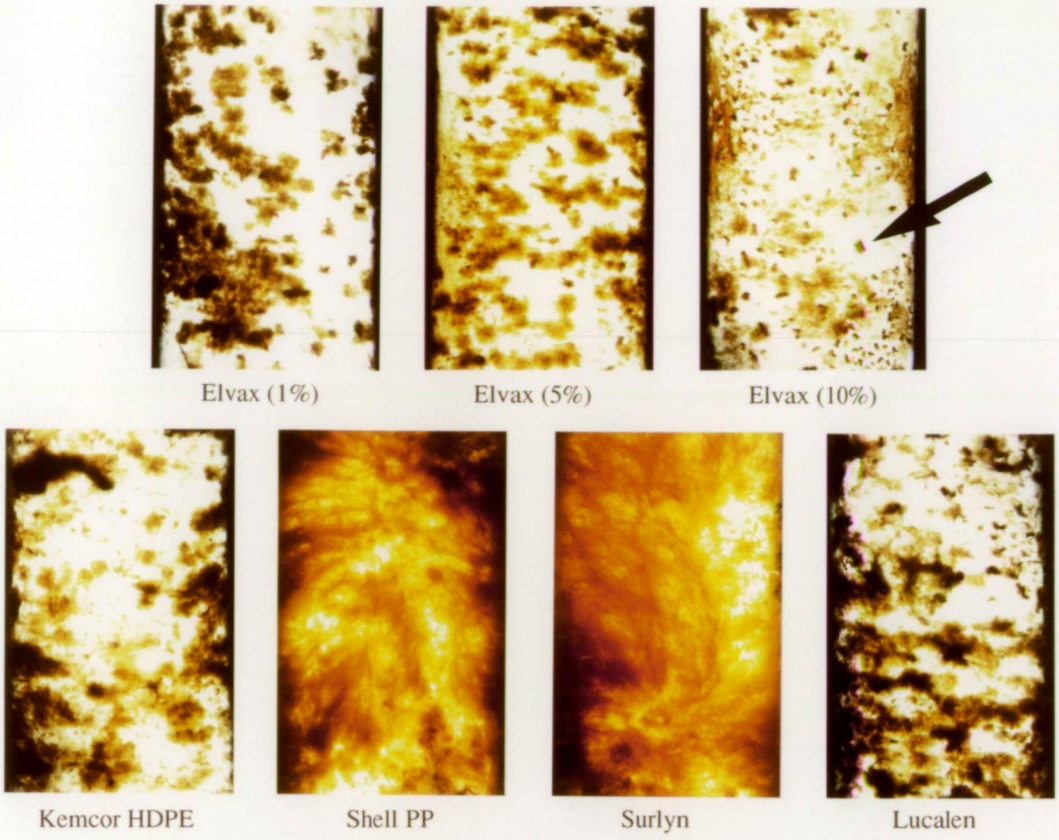


Figure 87. Polymers with the halogenated furanone after 47 days immersion. Tube-dwelling amphipods (arrow) were abundant on Elvax, 10% loading.

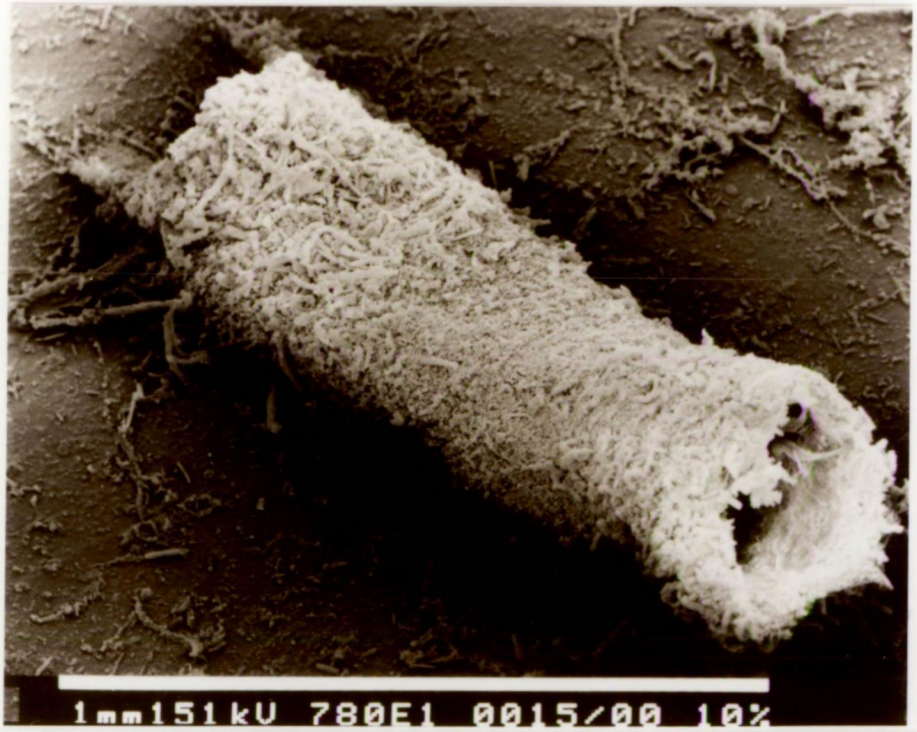


Figure 88. Tube built by a tube-dwelling amphipod on the surface of the polymer Elvax containing 10% halogenated furanone. Grazing by these amphipods greatly reduced the diatom fouling that surrounded each tube.

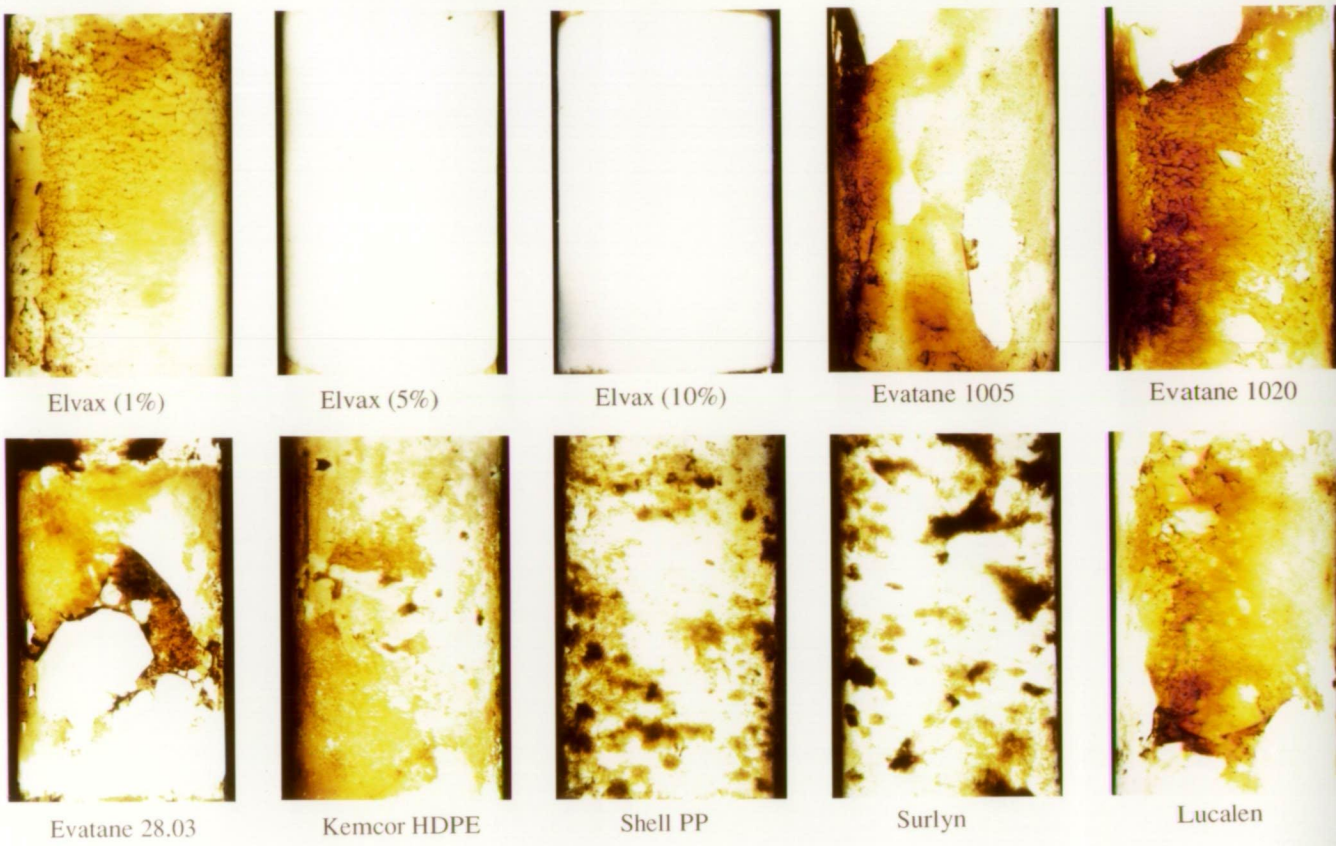


Figure 89. Polymers with Sea-nine 211 after 47 days immersion.

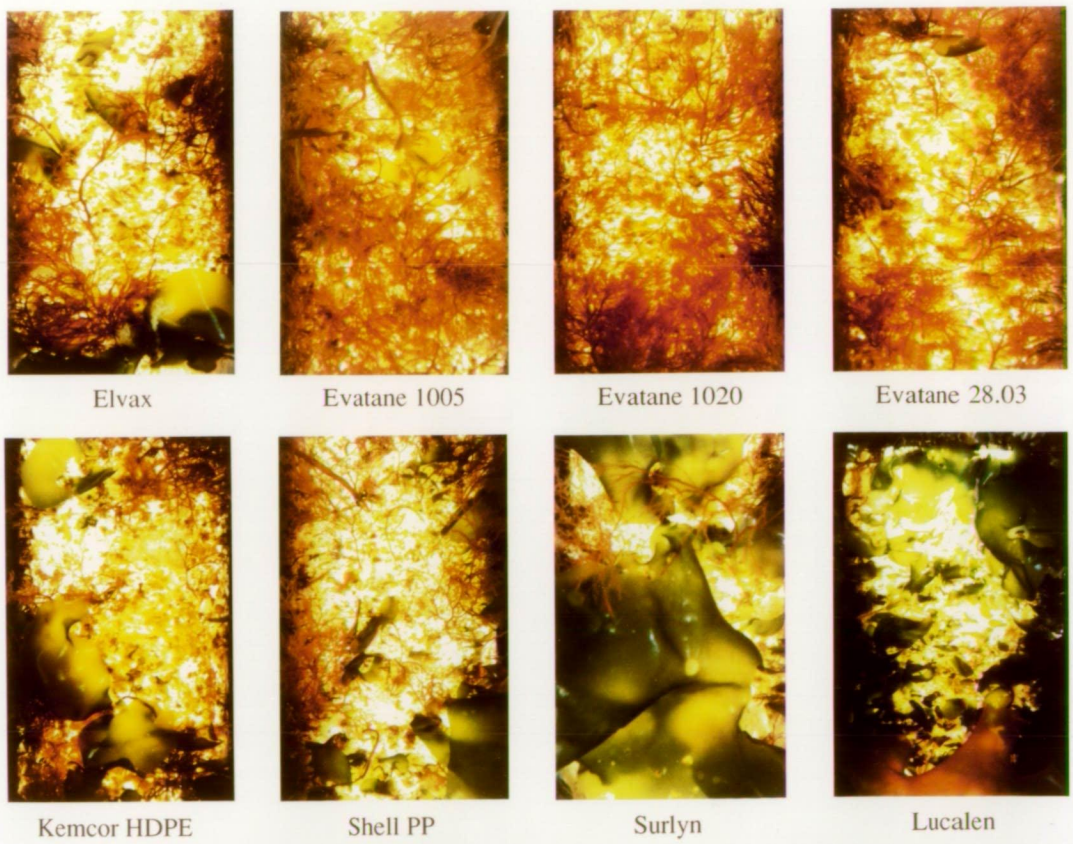


Figure 90. Control polymers after 110 days immersion.

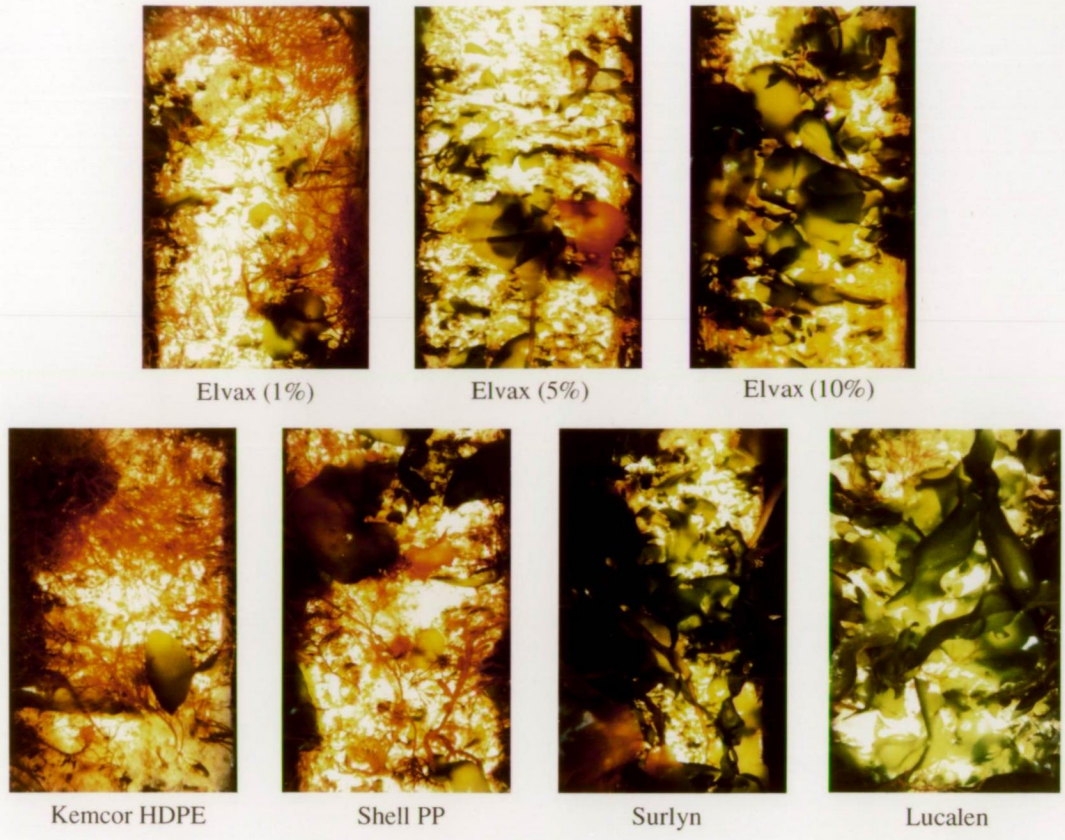


Figure 91. Polymers with the halogenated furanone after 110 days immersion.

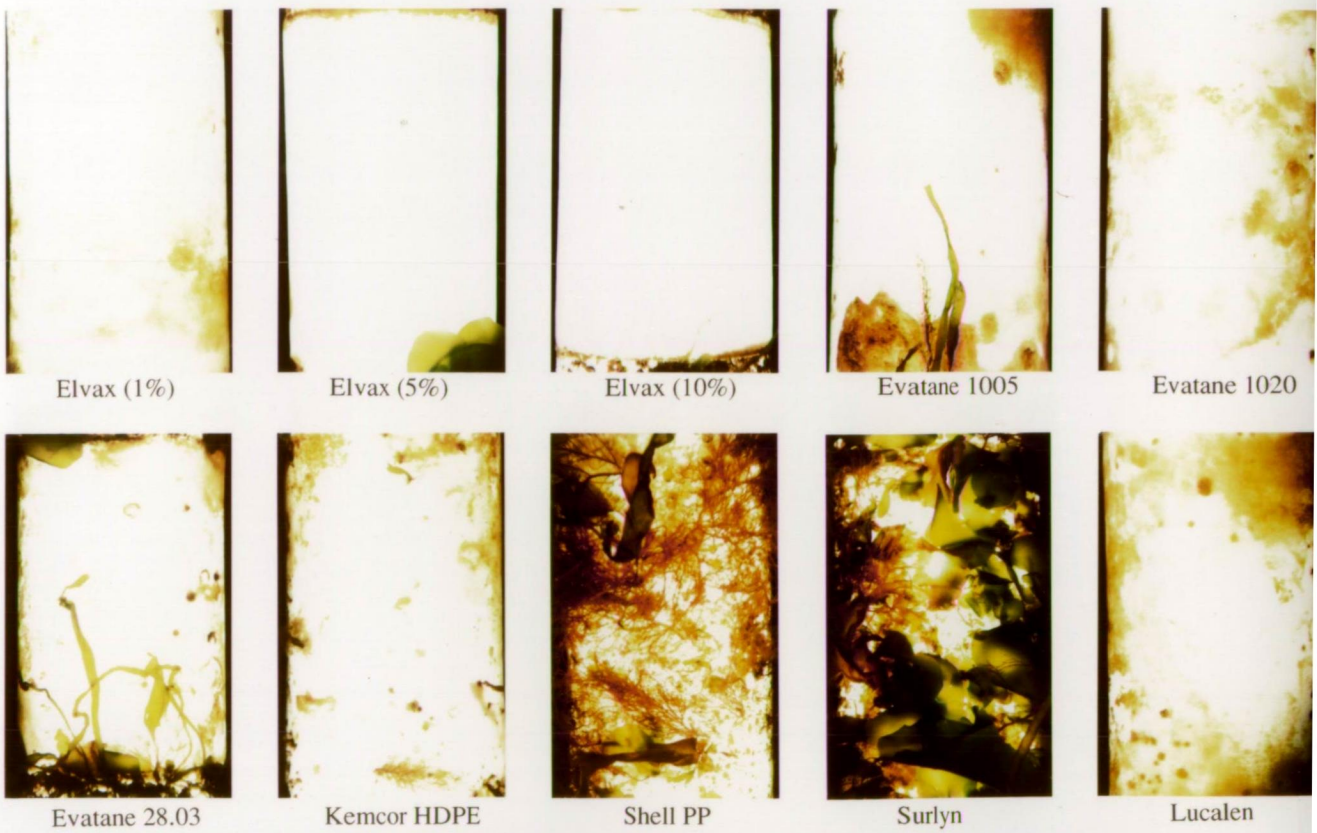


Figure 92. Polymers with Sea-nine 211 after 110 days immersion.

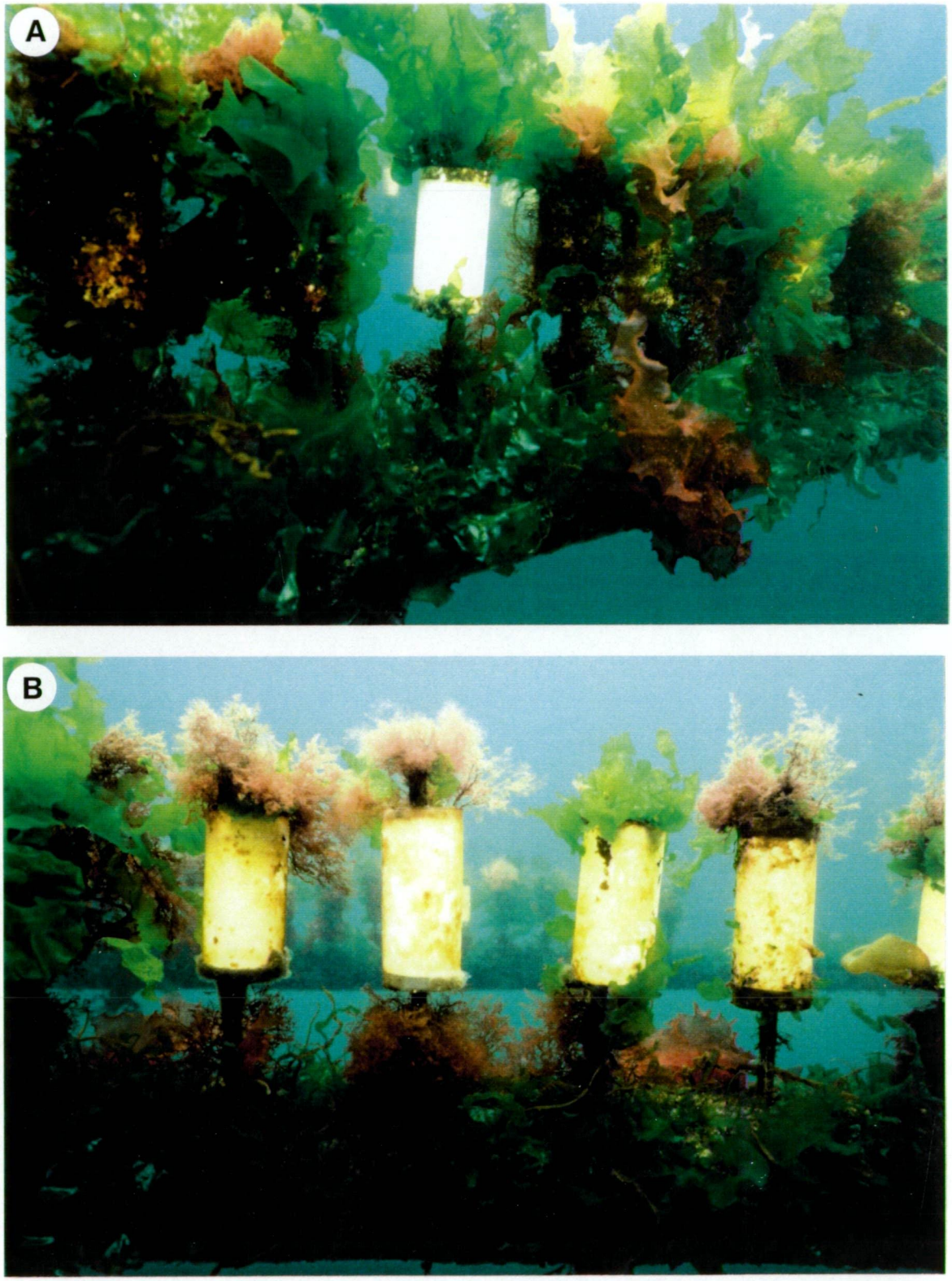


Figure 93. Fouling growth after 125 days immersion (September 8).
A: the central panel is 10% Sea-nine 211 in Elvax. The surrounding polymers are controls.
B: the four central panels all contain Sea-nine 211, and are (from left to right) Evatane 1020, Lucalen, Kemcor HDPE and Evatane 28.03. All four panels had a diatom film, but macrofouling was rare or absent.

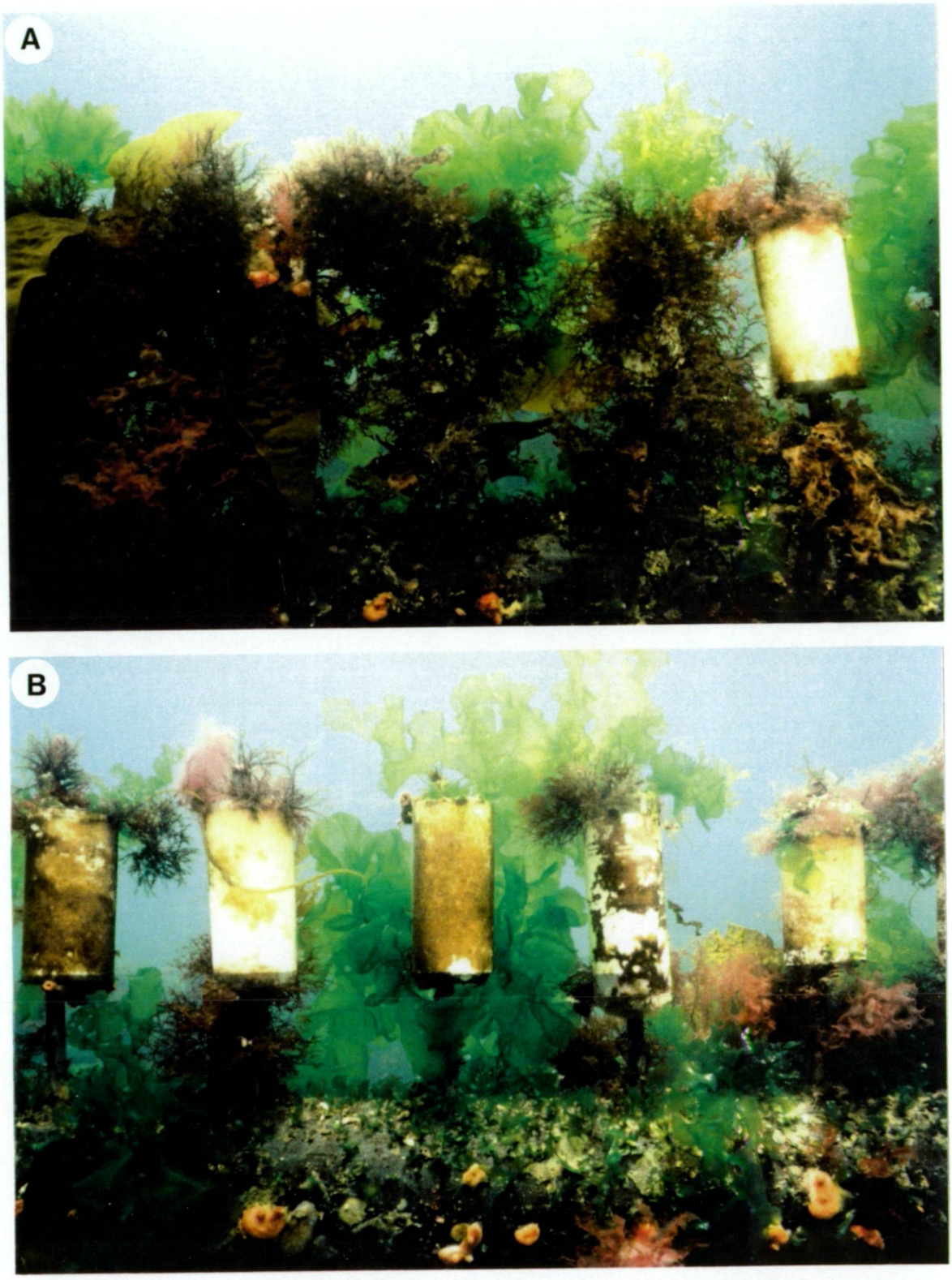


Figure 94. Fouling growth after 209 days immersion (December 1). Panels are the same a shown in figure 93.
A: the unfouled panel is 10% Sea-nine 211 in Elvax. The adjacent polymers are controls.
B: the five panels all contain Sea-nine 211, and are (from left to right) Evatane 1020, Lucalen, Kemcor HDPE and Evatane 28.03 and Elvax (1%). All five panels had a diatom film, but macrofouling was rare or absent.

Table 16. Field performance of antifouling polymers. Polymers were immersed on May 6, 1996, and the trial was completed on December 1, 1996 (209 days immersion). - = no obvious fouling.

	Days immersion										
	20	34	47	61	90	110	125	146	160	181	209
Controls											
Elvax 470	—— Diatom tufts ——						——— Macroalgae ———				
Evatane 1005	—— Diatom tufts ——						——— Macroalgae ———				
Evatane 1020	—— Diatom tufts ——						——— Macroalgae ———				
Evatane 28.03	—— Diatom tufts ——						——— Macroalgae ———				
Kemcor HDPE	—— Diatom tufts ——						——— Macroalgae ———				
Shell PP	—— Diatom tufts ——						——— Macroalgae ———				
Surlyn® 1707	—— Diatom tufts ——						——— Macroalgae ———				
Lucalen® A	—— Diatom tufts ——						——— Macroalgae ———				
Sea-nine 211											
Elvax 470 (1%)	-	———				Diatom film		———			
Elvax 470 (5%)	-	-	-	-	-	-	-	-	-	-	D.film
Elvax 470 (10%)	-	-	-	-	-	-	-	-	-	-	D.film
Evatane 1005	-	———				Diatom film		———			
Evatane 1020	-	———				Diatom film		———			
Evatane 28.03	-	———				Diatom film		———			
Kemcor HDPE	-	———				Diatom film		———			
Shell PP	D.film	—— Diatom tufts ——					——— Macroalgae ———				
Surlyn® 1707	D.film	—— Diatom tufts ——					——— Macroalgae ———				
Lucalen® A	-	———				Diatom film		———			
Halogenated furanone											
Elvax 470 (1%)	D.film	—Diatom tufts—					——— Macroalgae ———				
Elvax 470 (5%)	-	D.film	D.tufts				——— Macroalgae ———				
Elvax 470 (10%)	-	—Diatom film—		D.tufts			——— Macroalgae ———				
Kemcor HDPE	—— Diatom tufts ——						——— Macroalgae ———				
Shell PP	—— Diatom tufts ——						——— Macroalgae ———				
Surlyn® 1707	—— Diatom tufts ——						——— Macroalgae ———				
Lucalen® A	D.film	—Diatom tufts—					——— Macroalgae ———				

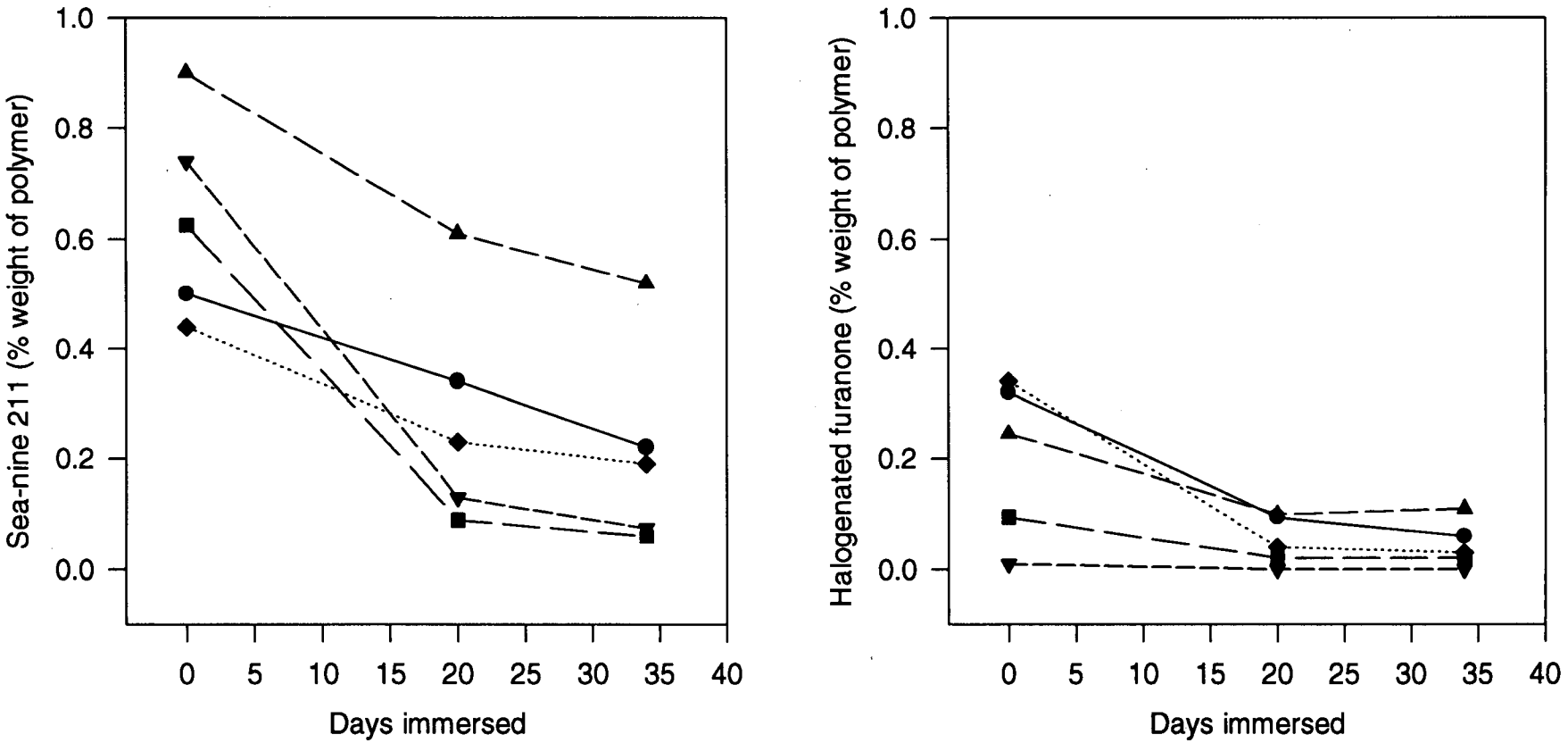


Figure 95. Quantities of antifoulant remaining in different types of polymer during field exposure. Each point is the mean of 4 samples.

—●— Elvax 470; —■— Kemcor HDPE; —▲— Shell PP; —▼— Surlyn 1707; ···◆··· Lucalen A

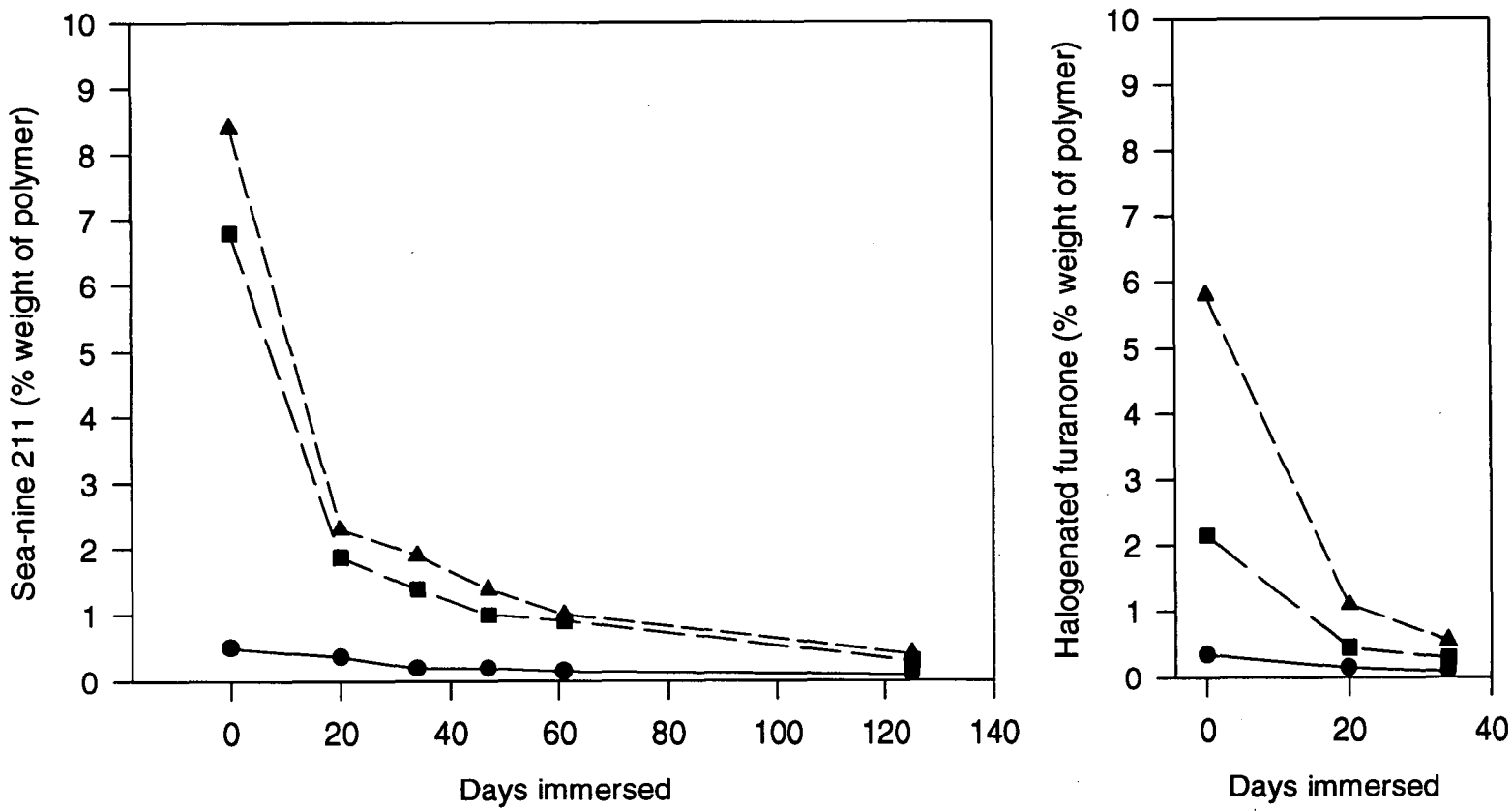


Figure 96. Quantities of antifoulant remaining in Elvax 470 during field exposure. Each point is the mean of 4 samples.
—●— 1% loading; —■— 5% loading; —▲— 10% loading

5.4 Discussion

The present study reports on the antifouling efficacy of four commercial biocides, two crude algal extracts, and an analog of an algal compound, when combined with extruded polymers. The primary aim of the project is to produce antifouling materials based on non-toxic, settlement-inhibiting compounds isolated from marine organisms. However, trials with commercial biocides were necessary to provide a benchmark for comparison of natural antifoulants and to identify the potential for extruded plastics as carriers of antifouling compounds. Further, Sea-nine 211 is claimed to rapidly degrade in seawater (< 24 hours) and not to bioaccumulate (Rohm & Haas data sheets, Appendix 1), and so was considered a potential antifoulant for mariculture.

Of the four commercial biocides, Sea-nine 211 and Irgarol 1051 are currently used as supplements to extend the life-time of copper-based antifoulants. This has been done to produce coatings of similar performance to the tributyltin antifoulants which are now banned for small boats in many countries (Dahl & Blanck, 1996). The products Busan 11-M1 and Nopcocide are broad spectrum microbiocides that are incorporated into exterior and interior building paints (as is Irgarol 1051) to prevent fungal and algal growth. The products Nopcocide N-96, Sea-nine 211 and Irgarol 1051 have recently been proposed as international standards for the evaluation of natural antifouling compounds in laboratory bioassays (de Nys *et al.*, 1996).

The algae *Delisea pulchra* and *Laurencia rigida* produce a range of compounds with antifouling activity (de Nys *et al.*, 1995, 1996). Two compounds isolated from *L. rigida*, elatol and descholoroelatol, have shown significantly greater activity against settlement of the barnacle *Balanus amphitrite* than Nopcocide N-96, Irgarol 1051 and Sea-nine 211 (de Nys *et al.*, 1996). Furthermore, these compounds are also more effective against settlement of the bryozoan *Bugula neritina* than Nopcocide N-96 and Irgarol 1051. However, these compounds are active through toxicity and are therefore limited in their suitability for environmentally-acceptable antifouling.

D. pulchra produces a range of halogenated furanones and en-ones which are active against algae, invertebrates and bacteria (de Nys *et al.*, 1993; de Nys & Steinberg, 1994; de Nys *et al.*, 1995). These are highly active at low concentrations (1-10 ng/ml), and effectively inhibit settlement without toxicity. These concentrations are an order of magnitude lower than copper and comparable to, or better than, effective concentrations of Nopcocide N-96, Irgarol 1051 and Sea-nine 211 in laboratory bioassays (de Nys *et al.*, 1995, 1996).

Polymers incorporating Sea-nine 211 were the most effective treatment in the present study (Figs 73, 94). Sea-nine 211 has previously been shown to affect a broad range of fouling taxa (Price *et al.,* 1991; Rittschof *et al.,* 1994; Arrage *et al.*, 1995; Vasishtha *et al.,* 1995), and is more effective than Irgarol 1051 and Nopcocide N-96 against growth of *Vibrio fischeri,* settlement of the bryozoan *B. neritina,* and settlement and germination of spores of the alga *Ulva lactuca* (de Nys *et al.,* 1996). Vasishtha *et al.* (1995) recommended a minimum effective release rate (MERR) of 5-7 $\mu g/cm^2$/day to prevent fouling by most taxa, with the exception of diatoms (15 $\mu g/cm^2$/day) and barnacles (20 $\mu g/cm^2$/day). The tolerance of diatoms may result from a barrier formed by their mucilage, as is the case with copper antifoulants (Brown *et al.*, 1988; Robinson *et al.*, 1992). However, the high recommended MERR for barnacles seems unlikely, as the barnacle *B. amphitrite* is equally susceptible to Sea-nine 211 as other fouling organisms (de Nys *et al.*, 1996). Vasishtha *et al.* (1995) correlated laboratory release-rate measurements with fouling on field-trialed panels and it is probable that field release rates differed from laboratory release rates, particularly after the development of a diatom film. Diatom tolerance was also demonstrated in the current study (eg. Figs 89, 94) but, although concentrations were not great enough to prevent their settlement, the release rates (even across a diatom film) were sufficient to prevent macrofouling. This is ideal for mariculture, because diatom fouling is not a concern, and the presence of diatoms indicates that release rates are not excessive, which ensures the greatest life-time.

Polymers incorporating *D. pulchra* compounds displayed good antifouling activity (Figs 72, 84), and both trials demonstrated that the EVA copolymer Dupont Elvax was suitable for containment and release of these compounds. The effective life time and release rates were dependent on the initial polymer loading (Fig. 96, Table 16). There are few publications of field trials that also demonstrate the ability of natural extracts to function as antifoulants in coatings or materials. Short-term trials (< 30 days) have demonstrated that sponge extracts significantly reduce fouling when incorporated into paints (Willemsen & Ferrari, 1993) and Phytagel™ (Henrikson & Pawlik, 1995). Longer-term trials (30 to 160 days) have shown Octocoral extracts significantly reduced fouling when microencapsulated and then included in polymeric coatings (Price *et al.*, 1992). Short-term trials have also demonstrated antifouling efficacy from coatings with natural product analogs (Price *et al.*, 1992; Clare *et al.*, 1995). However, these latter trials also show that coating life-time and efficacy is improved when analogs are combined with Sea-nine 211 (Clare *et al.*, 1995), or when techniques such as microencapsulation are used to

stabilise rapidly-degraded compounds (Price *et al.*, 1992). Consequently, whilst short-term efficacy is promising in the current study, there are many issues which must be considered to produce materials with a suitable life-time for a commercial product.

The effective concentration of an antifoulant varies between fouling taxa. In some cases the mode of action for an antifoulant restricts its activity to specific groups. For example, Irgarol 1051 is a herbicide that inhibits photosynthetic electron transport in chloroplasts (Dahl & Blanck, 1996), and has limited or no effect on invertebrates (de Nys *et al.*, 1996). Consequently, polymers with Irgarol 1051 were primarily colonised by hydroids. Algae preferentially grew on the surface of the hydroids rather than on the polymer (Fig. 74). Polymers with *L. rigida* extract developed greater algal fouling than controls (Fig. 72) and, as elatol and descholoroelatol are most active against invertebrates (de Nys *et al.*, 1996), this probably shows poor algal inhibition and effective prevention of invertebrate colonisation and grazing. Similarly, the abundance of tube-dwelling amphipods on polymers with the halogenated furanone (Figs 87, 88) may demonstrate a limit to the activity spectra of *D. pulchra* compounds (de Nys *et al.*, 1995). The preponderance of amphipods may indicate an increase in suitable food or reduced competition, because the abundance of tubes increased with analog concentration whereas tufts of diatoms (rather than films) decreased. However, it is also possible that *D. pulchra* compounds, which deter settlement via chemical signalling rather than via toxicity, may be ineffective against invertebrates that do not adhere to the substrate.

An important criterion for any antifouling coating is the long-term maintenance of an effective release rate (de la Court, 1988; Hunter & Evans, 1991). Coating failure (assuming the compound inhibits all fouling) will result from either the compound being completely leached from the system, or a reduction in release rate over time. The latter effect can result from characteristics of the coating or from a coating-compound interaction, or from biological and environmental conditions (Foster, 1994). The release rate from some coatings can be a function of flow rate over the surface and under stationary conditions it is reduced (de la Court, 1988). An increase or decrease in water temperature will also cause a corresponding change in leach rate for some coatings (Foster, 1994). When microbial biofilms develop they can act as barrier to toxin release (Bishop *et. al.*, 1974; Dempsey, 1981; Mihm & Loeb, 1988; Woods *et al.*, 1988). The antifouling performance of the polymer sheets in the current study should, therefore, not be interpreted as a function of antifoulant activity alone, but also of release rates, initial antifoulant concentration and conditions such as biofilm formation (eg. Figs 77-78).

The migration of an antifouling compound from within a polymer to the seawater interface is dependent on the diffusivity of the compound within the polymer (Weisman *et al.*, 1992). The resistance to diffusion will depend on the similarity of the compound and polymer (eg. their hydrophobicity and pH) and interactions between any functional groups of the compound and polymer. In the current study the antifoulant release rate was affected by polymer type (Fig. 95) and by the compound concentration (Fig. 96). Each polymer displayed a high initial release rate over the first 20 days (Fig. 95). Polymers incorporating the halogenated furanone had low initial concentrations and after 20 days immersion most had low levels of compound remaining and low release rates (Fig. 95). The lower initial concentrations of the furanone are likely to have resulted from degradation of this heat-sensitive compound under extrusion temperatures (greater than 100°C). In comparison, Sea-nine 211 occurred at a greater initial concentration in all polymers and after 20 days immersion greater levels of it remained and the release rates were higher. The only polymers with effective long-term inhibition of a diatom film were the 5% and 10% Sea-nine 211 treatments (Fig. 89), and these had the greatest concentrations and release rates (Fig. 96). As the release rates appear to be a function of compound concentration within the polymer (as has been proposed for other polymer systems; Weisman *et al.*, 1992), the results for the furanone are inconclusive because of the low concentrations that remained after the high initial release (Fig. 95). However, the antifouling performance of all polymers is not explained by their relative release rate. The Shell PP had the greatest initial concentration of Sea-nine 211, and a similar release rate to Elvax and Lucalen, but had poor antifouling efficacy (Fig. 85). This may indicate a greater rate of compound dissolution from the polymer into seawater, and therefore a lower concentration at the surface.

As part of this research, the shape of test panels was modified to increase their susceptibility to rapid colonisation by local fouling species, and to reduce inconsistencies in fouling distribution (eg. poor colonisation at panel centres). The impetus for this change was a study by Eashwar *et al.* (1991), which demonstrated that an increase in panel edge-to-centre ratio caused a corresponding increase in barnacle settlement. These authors further demonstrated that cylindrical panels, which were considered to have "all edges", were the most susceptible to settlement. Greater fouling on edges has been reported in other studies (eg. Schoener & Schoener, 1981; Okamura, 1986), and can be partly attributed to hydrodynamic effects, such as increased turbulence, which increase the chance of larvae and spores contacting a surface (Munteanu & Maly, 1981; Powlik,

1995). However, increased growth at edges is also likely to enable greater access to food for filter-feeding invertebrates or greater access to light for algae (Schoener & Schoener, 1981). In the present study, cylindrical panels developed the greatest fouling (Tables 14, 15), and fouling was more evenly distributed over their surface (Fig. 83; Table 13). Algae which had previously been observed to foul netting, such as *Polysiphonia infestans* and *Ceramium tasmanicum* (Chapter 3, Table 6), and *Lomentaria australis* and *Brongniartella australis* (Chapter 4, Table 7) were more abundant on cylindrical panels (Tables 14, 15). The only exception was *Polycerea nigrescens* which was more abundant on flat panels (Tables 14, 15), and which was not observed on netting. Cylindrical test panels have recently been used by other authors (eg. Rittschof *et al.*, 1992), and provide further benefits such as reduced orientation effects (Munteanu & Maly, 1981; Rittschof *et al.*, 1992). The usefulness of cylinders could be improved further by mounting them horizontally, so that light and orientation conditions select for two distinct communities, dominated by algae and invertebrates (Dahlem *et al.*, 1984).

In summary, polymers incorporating either a commercial short-lived biocide or a natural-product analog were effective antifouling treatments in field trials. The efficacy of the polymers was dependent on polymer type and the initial concentration of the antifouling compound. It is fortunate that both the natural-product analog and Sea-nine 211 performed well in the same polymer, as it permits future trials with a combination of the two. Rittschof *et al.* (1994) found that combinations of Sea-nine 211 and the natural-product analog γ decalactone were less toxic, but far more effective at settlement inhibition, than either compound alone.

CHAPTER 6

General discussion

Rapid biofouling of salmon-cage netting in Tasmania, Australia, jeopardises the health of the cultured species, and results in significant expenditure on labour and equipment for the frequent changing and cleaning of nets (Hodson & Burke, 1994; Lewis, 1994b). Whilst copper-based antifoulants are available for netting, they are undesirable to this industry because of concern over effects to the fish, and because the industry wishes to maintain its marketing image that fish are produced from clean, unpolluted waters (Lewis, 1994b). Furthermore, most large industries that rely heavily on coastal resources are under increasing pressure (from the community and government) to demonstrate responsible use of the environment. One of the major marine pollution issues over the past decade has been the wide-spread damage to coastal ecosystems, and aquaculture, from tri-*n*-butyltin antifoulants (Lewis, 1988; Alzieu, 1991). More recently, other toxic antifouling compounds, such as copper and Irgarol 1051, have also been found to persist in the environment and affect non-target species (Gough *et al.*, 1994; Dahl & Blanck, 1996; Tolosa *et al.*, 1996; Readman, 1996).

This thesis reports on a series of trials used to develop and evaluate environmentally acceptable fouling control strategies for fish cages. Three alternatives to traditional toxic antifouling were investigated, *viz.* (1) underwater cleaning, (2) a "foul-release" silicone coating, and (3) extruded polymers incorporating naturally-occurring compounds or commercial short-lived biocides. Each of these strategies was found to be applicable and effective for the prevention and/or reduction of fouling on netting. Trials identified future directions to improve antifouling efficacy, and potential problems such as resistant organisms. The development of a quantification technique and the description of fouling in each trial also provided information on the composition and variability within fouling communities on netting.

6.1 Characteristics of fouling communities on salmon-cage netting in Tasmania

Fouling was found to develop rapidly on netting at salmon farms in Tasmania, and often reduced the open mesh area by greater than 35% within a 14 day period (eg. Figs 7, 13, 34). Macroalgae were typically the most significant cause of rapid mesh occlusion at the depths studied (< 4 m) (eg. Figs 8, 14, 16). Hydroids (*Obelia australis* and *Syncoryne* sp.) (Figs 25, 9) and tube-dwelling amphipods (Fig. 5) were the only invertebrates found to rapidly develop into sizeable communities. The dominance of these invertebrates and algae as the most problematic fouling species separates finfish mariculture from other marine industries. Barnacles, bivalves and colonial invertebrates are often the major fouling organisms on ship hulls and oil platforms, and this reflects a difference in substrate characteristics and the longer immersion periods.

Twenty-two species of macroalgae were commonly found in the fouling communities (Table 17). Many species (eg. *Polysiphonia infestans, Ulva rigida, Enteromorpha prolifera, Scytosiphon lomentaria*) are cosmopolitan, and occur in fouling communities on a variety of surfaces (see Woods Hole, 1952; Fletcher, 1980; Lewis, 1982). Tube-dwelling diatoms were also a significant type of algal fouling (eg. Fig. 73), and have been reported as fouling organisms on salmon cages and netting panels in the USA (Moring & Moring, 1975; Huguenin & Ansuini, 1980).

Table 17. Common fouling algae on salmon-cage netting in Tasmania.

Rhodophyceae	Phaeophyceae	Chlorophyceae
Antithamnion sp.	*Asperococcus bullosus*	*Enteromorpha paradoxa*
Brongniartella australis	*Cutleria multifida*	*Enteromorpha prolifera*
Ceramium tasmanicum	*Ectocarpus* sp.	*Enteromorpha* sp.
Ceramium excellens	*Hincksia granulosa*	*Ulva rigida*
Champia affinis	*Scytosiphon lomentaria*	*Ulva lactuca*
Chondria fusifolia		
Griffithsia monilis		
Haraldiophyllum sinuosum		
Lomentaria australis		
Polysiphonia brodiaei		
Polysiphonia infestans		
Porphyra lucasii		

Invertebrates were rarely a significant cause of mesh occlusion relative to algal fouling. Other than hydroids and tube-dwelling amphipods, the only abundant invertebrates were caprellid amphipods, but these rarely caused significant mesh occlusion (Fig 10). Bryozoa were observed rarely, and occurred at far lower biomass than algae (Table 6). Invertebrates were only a major fouling organism on netting immersed for long periods. In this case, solitary ascidia became dominant (Figs 59, 66, Table 7) and added considerable weight to the netting (Fig. 65). However, invertebrates may become more of a problem if antifouling strategies are only directed at algae, and Huse *et al.* (1990) reported that shading of cages greatly reduced algal fouling but increased hydroid fouling.

Species resistant to each method of fouling control were observed during the field trials. Underwater cleaning selected for species capable of rapid regrowth (eg. *Antithamnion*, Fig. 29) and species that lay flat against the netting (eg. *Scytosiphon lomentaria*, Fig. 46). The commercial silicone coating selected for the algae *Ulva rigida* and *Polysiphonia brodiaei* (Table 7), probably because these species were better able to attach to this surface. Polymers incorporating commercial biocides and natural products selected for a range of species, depending on the activity spectrum of each compound (eg. Figs 72, 74, 87, 88). Previous studies have also described the emergence of resistant species. For example, copper-based antifoulants are well known to select for resistant macroalgae such as *Enteromorpha* and *Ectocarpus*, and microalgae such as *Amphora* and *Amphiprora* (Hall *et al.*, 1979; Callow, 1986). Underwater cleaning has been shown to select for species capable of rapid regrowth and recovery from fragmentation (Marsland, 1975). The development of resistant communities highlights the need for fundamental biological research of fouling species to identify: adhesion mechanisms, reproductive strategies and susceptibility to inhibitory compounds. Furthermore, research is also required to determine the mode of action for many antifouling strategies. The mechanism by which silicone elastomers reduce adhesion is not fully understood, and significant differences in performance have been reported for coatings of similar surface energy (Nevell *et al.*, 1996). The mechanisms of algal and larval settlement inhibition by natural compounds, and how structural changes affect their efficacy, also require investigation (Clare, 1996).

Large spatial and temporal variation was typical of the communities within cages. Significant differences in occlusion were measured between depths (Figs 7, 17), within adjacent areas on cage sides (Figs 17, 43C, D) and between cage sides (Figs 34-35). Also, the composition and occlusion of mesh varied greatly between small samples (Figs 16,

17). Short-term temporal variation (hours) resulted from movement of amphipods (Figs 11, 12), and significant weekly variation occurred from rapid fouling growth (Fig. 7). This variation confirms the importance of adequate replication in the description of fouling communities on non-toxic surfaces, a weakness of many previous studies of fouling on netting. Few studies have been conducted where conclusions were based on statistical analysis, and it is only because of gross differences in the results that authors have suggested effects (eg. Hodson & Burke, 1994). Only 1 replicate per treatment has been used in many panel studies (eg. Milne & Powell, 1967; Rothwell & Nash, 1977; Cheah & Chua, 1983), and only 1 replicate sample per treatment (eg. depth or cage side) has often been taken in studies of communities on cages (eg. Moring & Moring, 1975; Lee *et al.*, 1985). The importance of replicate panels for the accurate description of fouling communities has been discussed in previous studies (eg. Schoener & Greene, 1980; Schoener, 1984). Whilst replication is often limited by logistic and economic constraints, too few replicates greatly reduces statistical power to detect significant effects. Furthermore, when significant differences are observed between poorly replicated treatments, and the variability of the populations is not known, it is difficult to confidently separate a treatment effect from random effects.

It is now possible to sample large areas of netting rapidly, and achieve the level of replication required for fouling surveys, by image analysis of close-up underwater photographs. In chapter 3, 288 sample areas (each 16.0 cm by 10.7 cm) were photographed on each sampling day. The total number of samples was only restricted by the number of dives that could be physically conducted, however, sample numbers could be increased with multiple cameras and divers. Underwater photography is beneficial because it provides a permanent record of the fouling and allows dominant species to be identified and counted (eg. caprellids, Table 3). Because this method is non-destructive it is possible to conduct repeated-measures sampling so that temporal changes can be quantified at fixed locations (eg. Fig 7). The ability to measure fouling *in situ* is also advantageous, as repeated removal of the substrate from water may alter fouling communities (Schoener & Greene, 1981).

6.2 Strategies for fouling control on net cages

Fouling control methods applicable to fish-cage netting are restricted by cage design, netting design and the requirement to avoid deleterious effects to the cultured species. Antifouling treatments based on deterrent or toxic compounds must not cause adverse effects on the cultured species, such as reduced growth rates or reduced immuno-competency, and compounds must not bioaccumulate. Furthermore, the health and safety of farm staff who handle the nets must be considered. Netting material must be strong, long-lasting (at least 5 years), and non-abrasive to the fish. Modifications to cage design must be kept to a minimum if products are to be applicable to commercial finfish culture internationally.

Fouling control is made more difficult because of the nature of the substrate and environmental conditions. Multifilament netting is rough, non-toxic and has a high surface-area-to-volume ratio. The large crevices and many filaments of the netting are likely to aid colonisation, either through entrapment of suspended material or because larvae of some fouling invertebrates, and spores of common fouling organisms such as *Ectocarpus* and *Enteromorpha*, preferentially settle in depressions (Crisp, 1984). The 3-dimensional structure of mesh causes entanglement of drifting algae which are unaffected by antifoulants because they are not directly attached to the surface. Fouling growth is often rapid because the waters surrounding mariculture operations are enriched by organic and inorganic wastes (uneaten food, faecal and other excretory material) generated by the high-density fish populations (Gowen & Bradbury, 1987). Furthermore, spores and larval stages of fouling organisms are likely to be abundant at farms because cage systems constitute a massive surface area on which fouling communities reside.

Underwater cleaning of netting was found to be highly effective when there was good contact between the brushes and netting. Regular cleaning was found to keep mesh occlusion to a minimum, and to remove the majority of attached material (Section 3.4.2, Trial 2; Fig. 38). The cleaner was not intended to be a complete solution to biofouling, and is unable to operate on the outside or the bottom areas of a cage. Rather, it was designed to allow extended periods of net immersion. Provided that a number of biological and physical limitations are overcome, then it is likely that this strategy will be successful. The design of the cleaning head must be modified to ensure that the flexibility of netting does not reduce severity of scrubbing (Fig. 48). Reduced severity of scrubbing, which was caused because of poor contact between the brushes and netting, was the

greatest limitation to the current design. A system for extraction of cleaning debris is required to limit recolonisation of netting (Fig. 40) and avoid irritation of fish gills, dispersal of potential pathogens and organic pollution. One of the more difficult problems is the removal of residual structures of fouling organisms from crevices in the netting (eg. Figs 26-28, 39). This latter problem may be reduced by silicone coatings which would both fill crevices and facilitate the easy removal of fouling structures.

The application of a commercial silicone coating (Viridian 2000, International Paints) to netting was effective in increasing the ease of cleaning (Fig. 60) and reducing the total mass of attached fouling (Fig. 57). The silicone coating was particularly effective at preventing invertebrate fouling (Fig. 59), and the absence of solitary ascidians significantly reduced total fouling biomass (Figs 57, 65). On the silicone ascidians never exceeded 3 cm in length, suggesting that their adhesion strength was not sufficient to support weight and drag forces above this size. However, the total algal biomass on the silicone was comparable to that on uncoated netting (Fig. 58), and some algal species were more abundant on the silicone (*Ulva rigida* and *Polysiphonia brodiaei*). This latter effect was considered to represent effects of either surface colour, reduced competition, differences in attachment strength, or a combination of these. The greatest limitation to use of silicones in this industry is their poor abrasion resistance and tear strength (Milne & Callow, 1985), as it is likely that shore-based cleaning and handling would cause considerable coating damage. Nonetheless, the flexibility of silicone coatings and the effective reduction of fouling adhesion make them highly suitable for fish cages that will be cleaned *in situ*, and may provide an effective non-toxic solution to reduce the cost of fouling removal.

A promising area for development of long-term fish-cage antifouling is extruded polymers (which could be used for netting construction) that release either a commercial biocide such as Sea-nine 211 or compounds based on naturally-occurring halogenated furanones. Many polymer-compound combinations displayed excellent antifouling activity in field trials, and polymers incorporating Sea-nine 211 were fouled only by diatom slimes after 209 days immersion. Effective fouling prevention was shown to be a function of the polymer type and initial loading of the antifouling compound. Ethylene-vinyl acetate (EVA) copolymers were effective carriers for both Sea-nine 211 and halogenated furanones. A high-density polyethylene (Shell, HET 6100) and a copolymer of ethylene and acrylic acid (BASF, Lucalen® A) were also effective with Sea-nine 211. The effective release of both halogenated furanones and Sea-nine 211 from EVA copolymers may allow

the incorporation of mixtures of these compounds. Rittschof *et al.* (1994) found that combinations of Sea-nine 211 and the natural-product analog γ decalactone were less toxic, but far more effective for settlement inhibition, than either compound alone. The current success with polymers that incorporate antifouling compounds, and the many options for increasing their efficacy, are encouraging for the development of netting which resists fouling.

Each of the three fouling control strategies was successful for the prevention or reduction of fouling, and proffer environmentally-acceptable alternatives to toxic coatings. However, each strategy also had limitations; the underwater cleaning system could not be used on the entire cage surface, the silicone coatings lack abrasion resistance, and the construction of netting with antifouling polymers will not prevent entanglement of drifting algae. Effective control of fouling on mariculture cages is therefore likely to involve a combination of physical and chemical strategies, as is the case with many marine organisms (see Wahl, 1989). In many cases the future development of these strategies will depend on research into their mode of action, and also the characteristics of resistant organisms. Whilst the current research trials were targeted at antifouling of finfish cages, the results have wider implications because many other mariculture sectors (eg. pearl culture and scallop culture) also use netting for animal containment and require non-toxic antifouling.

6.3 Recommendations

There are several options currently available for fouling prevention on Tasmanian salmon cages. A reduction in the frequency of net changing, from every 2 weeks to every 2 months during spring and summer, is achievable in the near future. However, strategies that allow nets to remain immersed throughout the seasons of peak fouling, or for several years, will require further research.

- **Mechanical cleaning**

Development of an effective *in situ* cleaner is within the resources of the Tasmanian salmon industry, and the trials with the prototype suggest that this strategy would have a high likelihood of success. With regular *in situ* cleaning it would only be necessary to change nets (for onshore cleaning) when there is significant fouling at the base or outside of a cage. The primary obstacle for use of this strategy is the need to develop an alternative design for presentation of the brushes to ensure that there is sufficient contact between the bristles and netting. Once this is achieved, a system for removal of debris is required to prevent organic loading of the immediate environment. Furthermore, the debris could potentially irritate fish gills and may act to disperse pathogens that colonise the netting.

- **Silicone "foul-release" coatings**

Silicone coatings would be highly suitable for cages that will be cleaned *in situ*, and may allow a reduction in the severity of scrubbing needed for fouling removal (which would also reduce the power requirements for cleaning). Increased fouling removal would limit regrowth from residual fouling structures. The rate of recolonisation may also be reduced because the coatings fill crevices in the netting weave. The reduction in attached biomass of many fouling taxa, particularly solitary ascidians, also suggests that silicones could reduce fouling of predator netting.

Silicone coatings are suitable for mariculture because they are non-toxic, and their flexibility makes them well suited for application to netting. However, their poor abrasion resistance makes them unsuitable for present onshore cleaning and handling methods. Consequently, whilst the use of silicones would be beneficial they can not be used without development of *in situ* cleaning equipment and/or modifications to onshore procedures.

- **Extruded antifouling polymers**

Extruded polymers were found to be suitable for the containment and release of antifouling compounds, and may offer a suitable alternative to metal-based antifouling

paints. Polymers incorporating Sea-nine 211 were highly effective, and these should be suitable for aquaculture as it is claimed that Sea-nine 211 rapidly degrades in seawater (< 24 hours) and does not bioaccumulate (Rohm and Haas data sheets, Appendix 1). Polymers incorporating a natural-product analog (a halogenated furanone) showed good short-term antifouling, however, the long-term performance could not be evaluated because of low initial concentrations. Halogenated furanones are ideal for mariculture because they are active at low concentrations and effectively inhibit settlement without toxicity (de Nys et al., 1995).

The results to date are promising, but this project is clearly at an early stage, and future trials are required to evaluate the furanones in higher concentrations in a range of polymers. Combinations of the furanone with Sea-nine 211 should also be evaluated as a method to reduce the toxicity of Sea-nine 211 polymers. Prior to commercialisation of this technology ecotoxicological trials are required to evaluate any deleterious effects, such as reduced growth rates or immuno-competency, that these compounds may cause to the cultured species. Provided that the compounds do not affect the cultured species, nor bioaccumulate, then netting constructed from extruded polymers may provide a long-term (up to 5 years) solution to fouling prevention on net cages.

REFERENCES

Aarsnes J V, Rudi H, Loland G (1990) Current forces on cages, net deflection. *Engineering for Offshore Fish Farming.* Thomas Telford, London, pp 137-152

Alabaster J S, Lloyd R (1982) Ammonia. In: Alabaster J S, Lloyd R (eds) *Water Quality Criteria for Freshwater Fish*, Butterworths, 279 pp

Alberte R S, Snyder S, Zahuranec B J, Whetstone M (1992) Biofouling research needs for the United States Navy: program history and goals. *Biofouling* **6:** 91-95

Alzieu C (1991) Environmental problems caused by TBT in France: assessment, regulations, prospects. *Mar Environ Res* **32:** 7-17

Amsler C D (1984) Culture and field studies of *Acinetospora-crinita* Carmichael Sauvageau Ectocarpaceae Phaeophycaea in North-Carolina USA. *Phycologia* **23:** 377-382

Anon (1979) Rotating cage in Scottish trials. *Fish-Farmer* **3:** 14

Anon (1993) How Ocean Spar solves problems of currents. *Fish Farm Int* **21:** 20

Anon (1994) New machine cleans nets on the cages. *Fish Farm Int* **21:** 22

Anon (1996a) New protection by Bio for cage nets. *Fish Farm Int* **23:** 44

Anon (1996b) 'First' to protect cage nets against fouling. *Fish Farm Int* **23:** 26

Anon (1996c) Antifoulants presented at Kobe exhibition. *Fish Farm Int* **23:** 29

Anon (1996d) Antifouling saves on net handling. *Fish Farm Int* **23:** 19

Anon (1996e) *Marine farming development plan, Huon River and Port Esperance.* October 1996, Marine Resources Division, Department of Primary Industry and Fisheries, Tasmania, 81 pp

ANZECC (Australian and New Zealand Environment and Conservation Council) (1995) *Maritime Accidents and Pollution: Impacts on the Marine Environment from Shipping Operations.* Paper for Public Comment, Commonwealth of Australia, 50 pp

Arakawa K Y (1973) Prevention and removal of fouling on cultured oysters - a handbook for growers. (Translated into English by R B Gillmore). *Marine Sea Grant Technical Report*, University of Maine, Orono, Maine **56:** 38 pp

Arrage A A, Vasishtha N, Sundberg D, Bausch G, Vincent H L, White D C (1995) On-line monitoring of antifouling and fouling-release surfaces using bioluminescence and fluorescence measurements during laminar flow. *J Ind Micro* **15:** 277-282

Baier R E, Meyer A E (1992) Surface analysis of fouling-resistant marine coatings. *Biofouling* **9:** 165-180

Bakus G J (1988) Practical and theoretical problems in the use of fouling panels. In: Thompson M F, Sarojini R, Nagabhushanam R (eds) *Marine Biodeterioration.* A A Balkema, Rotterdam, pp 619-630

Balls P W (1987) Tributyltin (TBT) in the waters of a Scottish sea loch arising from the use of antifoulant treated netting by salmon farms. *Aquaculture* **65**: 227-237

Becker K (1993) Attachment strength and colonisation patterns of two macrofouling species on substrata with different surface tension (in situ studies). *Mar Biol* **117:** 301-309

Ben-Zion M, Achituv Y, Stambler N, Dubinsky Z (1991) A photographic, computerized method for measurements of surface area in *Millepora. Symbiosis* **10:** 115-121

Beveridge M C M (1996) *Cage Aquaculture.* 2nd edition, Fishing News Books Ltd, Oxford, England. 352 pp

Bishop J H, Silva S R, Silva V M (1974) A study of microfouling on antifouling coatings using electron microscopy. *J Oil Col Chem Assoc* **57:** 30-35

Black E A , Gillis D J, Hay D E, Haegele C W, Levings C D (1992) Predation by caged salmon in British Columbia. *Proc 1992 Meet Aqua Assoc Canada* **92:** 58-60

Blair A, Burgess W H (1979) Rotating floating cage for fish culture simplifies cleaning and replacement of panels. *Prog Fish-Cult* **41:** 74-76

Blair A, Campbell R, Grant P T (1982) A submersible fish cage that can be rotated on the surface to remove biofouling and for other purposes. *Aquaculture* **29:** 177-184

Boyd C E (1982) Water quality management for pond fish culture. *Developments in Aquaculture and Fisheries Science*, Vol 9, Elsevier, Amsterdam, 318 pp

Branson E (1993) Environmental aspects of aquaculture. In: Brown L (ed) *Aquaculture for Veterinarians*, Pergamon Press, Oxford, pp 57-67

Brault D, Quéguiner B (1989) Effect of inorganic and organic nitrogen sources on growth and *Ulva gigantea* (Kutzing) Blinding. In: De Pauw N, Jaspers E, Ackefors H, Wilkins N (eds) *Aquaculture - A Biotechnology in Progress, Proc Int Conf Aquaculture Europe '87*, Amsterdam, Netherlands, June 2-5, 1987, pp 425-430

Brown L N, Robinson M G, Hall B D(1988) Mechanisms for copper tolerance in *Amphora coffeaeformis* – internal and external binding. *Mar Biol* **97:** 581-586

Bruno D W, Ellis A E (1988) Histopathological effects in Atlantic salmon, *Salmo salar* L., attributed to the use of tributyltin antifoulant. *Aquaculture* **72:** 15-20

Bultman J D, Griffith J R (1994) Fluoropolymer and silicone fouling-release coatings. In: Thompson M F, Nagabhushanam R, Sarojini R, Fingerman M (eds) *Recent Developments in Biofouling Control,* A A Balkema, Rotterdam, pp 383-389

Buchsbaum R, Buchsbaum M, Pearse J, Pearse V (1987) *Animals without backbones.* 3rd edition, University of Chicago Press, Chicago, 572 pp

Butler A J, Connolly R M (1996) Development and long term dynamics of a fouling assemblage of sessile marine invertebrates. *Biofouling* **9:** 187-209

Bwathondi P O J, Ngoile M A K (1982) Bivalves fouling floating cages used in aquaculture. *Indian J Mar Sci* **11**: 283-285

Bwathondi P O J (1985) The potential of marine polyculture in developing countries: a case study in Tanzania. In: Labish-Chao N, Kirby-Smith W (eds) *Proc Int Symp on Utilization of Coastal Ecosystems: Planning, Pollution and Productivity*, Rio Grande, Brazil, 21 Nov 1982. **1:** 313-320

Caillouet C W (1972) Rotatable cage for high-density aquaculture. *Prog Fish-Cult* **34**: 8

Callow M E, Pitchers R A, Santos R (1988) Non-biocidal anti-fouling coatings. In: Houghton D R, Smith R N, Eggins H O W (eds) *Biodeterioration 7*, Elsevier Applied Science Publishing Co., London, pp 43-48

Chaudhary B R, Srivastava N (1995) Rhizoid differentiation in the filamentous green alga *Spirogyra irregularis* Nageli (Chlorophycaea). *Cytobiosis* **8:** 181-187

Cheah S H, Chua T E (1979) A preliminary study of the tropical marine fouling organisms on floating net cages. *Malay Nat J* **33**: 39-48

Cheah S H, Chua T E (1983) The rate of encrustation of biofouling organisms on experimental floating net-cages in tropical coastal waters. *Proc Symp on Coastal Aquaculture*, Cochin, India, 12-18 January, 1980. Part 2: Molluscan Culture. *Mar Biol Assoc India*, no. 6, pp 677-683

Chengxing Z (1990) Ecology of ascidians in Daya Bay. *Collections of Papers on Marine Ecology in Daya Bay, 2.* pp 397-403

Christie A O (1972) Spore settlement in relation to fouling by Enteromorpha. In: Acker R F, Brown B F, DePalma J R, Iverson W P (eds) *Proc 3rd Int Congr Mar Corr & Foul*, Oct 2-6, 1972, National Bureau of Standards, Maryland, USA, pp 674-681

Christie A O, Shaw M (1968) Settlement experiments with zoospores of *Enteromorpha intestinalis* (L.) Link. *Br Phycol Bull* **3:** 529-534

Chua T E, Teng S K (1977) Floating fish pens for rearing fishes in coastal waters, reservoirs and mining pools in Malaysia. *Fisheries Bulletin of the Ministry of Agriculture of Malaysia*, 20

Clare A S (1996) Marine natural product antifoulants: status and potential. *Biofouling* **9:** 211-229

Clare A S, Rittschof D, Gerhart D J, Maki J S (1992) Molecular approaches to non-toxic fouling. *Invertebr Reprod Dev* **22:** 67-76

Clare A S, Rittschof D, Price R R, Gerhart D J (1995) Khellin, a natural product analog with antifouling activity: laboratory and field studies. In Bousher A, Edyvean R G J (eds) *Biodeterioration and Biodegradation*, 9. Institute of Chemical Engineers, pp 573-580

Cohen L, Fowler J (1990) Practical statistics for field biology. John Wiley & Sons, Chichester, 227 pp

Cologer C P, Bohlander G S, Preiser H S (1977) Review of underwater cleaning methods and their interaction on navy anti-fouling systems. *J Coatings Technology* **49:** 51-60

Conn A F (1980) Field trials of a cavitating-jet fouling removal device. In: *Proc 5th Int Congr Mar Corr & Foul*, Barcelona, Spain, 19-23 May, 1980, pp 39-53

Connell J H (1975) Some mechanisms producing structure in natural communities: a model and evidence from field experiments. In: Cody M L, Diamond J M (eds)

Ecology and Evolution of Communities. Belknap Press, Cambridge, Mass, pp 460-491

Crisp D J (1974) Factors influencing the settlement of marine invertebrate larvae. In: Grant P T, Mackie A M (eds) *Chemo Reception in Marine Organisms*. Academic Press, London, pp 177-265

Crisp D J (1984) Overview of research on marine invertebrate larvae, 1940-1980. In: Costlow J D, Tipper R C (eds), *Marine Biodeterioration: An Interdisciplinary Study*, Naval Institute Press, Maryland, pp 103-126

Cronin E (1995) An investigation into the effects of net fouling on the oxygen budget of southern bluefin tuna (*Thunnus maccoyii*) farms. Honours Thesis, University of Adelaide, South Australia, 72 pp

Dahl B, Blanck H (1996) Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms. *Mar Pollut Bull* **32:** 342-350

Dahlem C, Moran P J, Grant T R (1984) Larval settlement of marine sessile invertebrates on surfaces of different colour and position. *Ocean Sci Eng* **9:** 225-236

Davies I M, McKie J C (1987) Accumulation of total tin and tributyltin in muscle tissue of farmed Atlantic salmon. *Mar Pollut Bull* **18:** 405-407

Day R W (1977) Two contrasting effects of predation on species richness in coral reef habitats. *Mar Biol* **44:** 1-5

de la Court F H (1988) The minimum leaching rate of some toxins from antifouling paints required to prevent settlement of fouling organisms. In: Houghton D R, Smith R N, Eggins H O W (eds) *Biodeterioration 7*, Elsevier Applied Science Publishing Co., London, pp 305-308

Dempsey M J (1981) Colonisation of antifouling paints by marine bacteria. *Bot Mar* **24:** 185-191

de Nys, Wright A D, Konig G M, Sticher O (1993) New halogenated furanones from the marine red alga *Delisea pulchra (cf. fimbriata). Tetrahedron* **49:** 11213-11220

de Nys R, Steinberg P (1994) Antifouling compounds from the marine red alga *Delisea pulchra*. In: Belwood O, Choat H, Saxena N (eds) *Recent Advances in Marine Science and Technology '94*. Pacon International, James Cook University, pp 351-361

de Nys R, Steinberg P D, Willemsen P, Dworjanyn S A, Gabelish C L, King R J (1995) Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling* **8:** 259-271

de Nys R, Leya T, Maximilien R, Afsar A, Nair P S R, Steinberg P D (1996) The need for standardised broad scale bioassay testing: a case study using the red alga *Laurencia rigida. Biofouling* **10:** 213-224

Doedens R (1992) Mechanical cleaning of nets - Preliminary report. In: *Proc 1992 Research and Development Review Seminar*, 29th April, 1992, pp 203-206

Dubost N, Masson G, Moreteau J-C (1996) Temperate freshwater fouling on floating net cages: method of evaluation, model and composition. *Aquaculture* **143:** 303-318

Eashwar M, Maruthamuthu S, Manickam S T (1991) An assessment of preference for coupon positions by tropical marine fouling organisms. *Biofouling* **3:** 277-286

Edwards A, Edelston D J (1976) Marine fish cages - the physical environment. *Proc Royal Society Edinburgh* (B) **75**: 207-221

Fischer E C, Castelli V J, Rodgers S D, Bleile F R (1984) Technology for control of marine biofouling - a review. In: Costlow J D, Tipper R C (eds), *Marine Biodeterioration: An Interdisciplinary Study*, Naval Institute Press, Maryland, pp 261-299

Fletcher R L (1980) Marine fouling algae. *Catalogue of Main Marine Fouling Organisms*, ODEMA, Vol. 6, Algae, pp 7-15

Fletcher R L, Baier R E, Fornalik M S (1984a) The influence of surface energy on spore development in some common marine fouling algae. *Proc 6th Int Congr Mar Corr & Foul*, Greece, 5-8 September, 1984, pp 129-144

Fletcher R L, Jones A M, Jones E B G (1984b) The attachment of fouling macroalgae. In: Costlow J D, Tipper R C (eds) *Marine Biodeterioration: an interdisciplinary study,* Proceedings of the Symposium on Marine Biodeterioration, Annapolis, 20-23 April, 1981, Naval Institute Press, pp 172-182

Foster A C (1994) Current antifouling technologies. In: Kjelleberg S, Steinberg P (eds), *Biofouling: Problems and Solutions,* Proceedings of an International Workshop, The University of New South Wales, Sydney, Australia, pp 44-48

Fukuda H (1965) Report of an investigation on environmental parameters in relation to fish farming. Tokushima Prefectural Fisheries Experimental Station, pp 1-32

Geffen A (1979) Rotating fish cage to prevent fouling. *Aquaculture* **16:** 83-85

Gjovik J (1987) The state of salmonid farming in Tasmania. In: Hortle E (ed) *The Tasmanian aquaculture industry - Proc 1st Tasmanian Aquaculture Conference*, Dept Sea Fisheries, Tasmania, Tech Rep 20, pp 26-36

Gormican S J (1989) Water circulation, dissolved oxygen, and ammonia concentrations in fish net-cages. Masters Thesis. University of British Columbia, Canada, 62 pp

Gough M A, Fothergill J, Hendrie J D (1994) A survey of southern England coastal waters for the s-triazine antifouling compound Irgarol 1051. *Mar Pollut Bull* **28:** 613-620

Gowen R J, Bradbury N B (1987) The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr Mar Biol Ann Rev* **25:** 563-575

Gracey M T (1974) *Plan for development and evaluation of underwater cleaning techniques.* Marine Section, National Maritime Research Centre, Galveston, Texas, NMRC-273-32100-R2

Greenland D C, Newton S H, Faucette R F Jr. (1988) Effects of cage encrustation by the bryozoan *Plumatella casmiana* on production of channel catfish. *Prog Fish-Cult* **50**: 42-45

Gucinski H, Baier R E, Meyer A E, Fornalik M S, King R W (1984) Surface microlayer properties affecting drag phenomena in seawater. *Proc 6th Int Congr Mar Corr & Foul*, 5-8 Sep, 1994, Athens, Greece, pp 585-604

Gularte R C, Huguenin J E (1984) Mesh hydrodynamic data. *Design guide for use of copper alloy expanded metal mesh in marine aquaculture. INCRA Project 268B,* Woods Hole Engineering Associates Inc, pp 54-58

Haddad A M G, Ormond R F G (1994) Use of photography and image analysis for *in situ* monitoring of growth rates of intertidal fucoid algae. *J Mar Biol Ass UK* **74:** 727-730

Haegele C W, Miller D C, Brown T J, McCarter P B, Hay D E (1991) Availability of wild feed to salmon in aquaculture cages. *Can Manuscr Rep Fish Aquat Sci*, no. 2117, 38 pp

Hall A, Fielding A H, Butler M (1979) Mechanisms of copper tolerance in the marine fouling alga *Ectocarpus siliculosus* – evidence for an exclusion mechanism. *Mar Biol* **54:** 195-199

Harlin M M, J M Lindbergh (1977) Selection of substrata by seaweeds: optimal surface relief. *Mar Biol* **40:** 33-40

Harris L G, Irons K P (1982) Substrate angle and predation as determinants in fouling community succession. In: Cairns J (ed) *Artificial Substrates*. Ann Arbour Science, Mich, pp 131-174

Hasse J (1974) Utilisation of rabbit fishes (Siganidae) in tropical oyster culture. *Prog Fish-Cult* **36:** 160-162

Henrikson A A, Pawlik J R (1995) A new antifouling assay method: results from field experiments using extracts of four marine organisms. *J Exp Mar Biol Ecol* **194:** 157-165

Henschel J R, Branch G M, Cook P A (1990) The colonisation of artificial substrata by marine sessile organisms in False Bay. 2. Substratal material. *S Afr J Mar Sci* **9:** 299-307

Hisaoka M, Nogami K, Takeuchi O, Suzuki M, Sugimoto H (1966) Studies on sea water exchange in fish farms-II. Exchange of sea water in floating net. *Bull Naiki Regional Fisheries Research Laboratory [Japan]* **23**: 21-43, (contribution no. 115) (in Japanese)

Hodson S L (1994) In situ mechanical cleaning of netting: evaluation of the brush tester and development of an analysis technique. *SALTAS Research and Development Review Seminar*, 18 August, 1994, Tasmania, Australia, pp 227-238

Hodson S L, Burke C (1994) Microfouling of salmon-cage netting: a preliminary investigation. *Biofouling* **8**: 93-105

Holmstrom C, Kjelleberg S (1994) The effect of external biological factors on settlement of marine fouling invertebrates and new antifouling technology. *Biofouling* **8:** 147-160

Huguenin J E, Ansuini F J (1975) The advantages and limitations of using copper materials in marine aquaculture. *Ocean '75*. Conference record, Marine Technology Society, Institute of Electrical and Electronic Engineers, pp 444-453

Huguenin J E, Ansuini F J (1977) The design, construction, testing and evaluation of copper-based commercial prototype equipment for use in marine aquaculture. *Annual report INCRA project No. 268*, 61 pp

Huguenin J E, Ansuini, F J (1978) A review of the technology and economics of marine fish cages systems. *Aquaculture* **15:** 151-170

Huguenin J E, Ansuini F J (1980) The design, construction, testing and evaluation of copper-based commercial prototype equipment for use in marine aquaculture. *Annual report INCRA project No. 268* (January), 67 pp

Huguenin J E, Ansuini F J (1981) Marine biofouling of synthetic and metallic screens. Ocean 81 Conference Technical Record, Boston, MA, 545-549

Huguenin J E, Ansuini F J (1984) Marine biofouling of synthetic and metallic screens. *Design guide for use of copper alloy expanded metal mesh in marine aquaculture. INCRA Project 268B.* Woods Hole Engineering Associates Inc, pp 48-53

Hunter C J, Farr W E (1970) Large floating structure for holding adult Pacific salmon (*Oncorhynchus* sp.). *J Fish Res Bd Canada* **27**: 947-950

Hunter J E, Evans L V (1991) Raft trial experiments on antifouling paints containing zineb and copper. *Biofouling* **3:** 113-137

Huse I, Bjordal Å, Fernö A, Furevik D (1990) The effect of shading in pen rearing Atlantic salmon (*Salmo salar*). *Aqua Eng* **9**: 235-244

Inoue H (1972) On water exchange in a net cage stocked with the fish, Hamachi. *Bull Jap Soc Sci Fish* **38**: 167-176 (in Japanese)

Jungalwalla, P J (1991) The development of an integrated saltwater salmonid farming industry in Tasmania, Australia. In: R H Cook and W Pennel (eds) Proceedings of the special session on salmonid aquaculture, World Aquaculture Society, 16 Feb, 1989, Los Angeles, USA. *Can Tech Rep Fish Aquat Sci* **1831:** 65-73

Kadowaki S, Kasedo T, Nakazono T (1978) Continuous records of DO contents by cruising in the coastal culture farms. 1. Relation between DO content and fish density in cages. *Mem Fac Fish Kagoshima Univ* **27**: 273-280. (translated manuscript from *Can Transl Fish Aquat Sci*, 1985, no. 5189)

Kannan K, Tanabe S, Iwata H, Tatsukawa R (1995a) Butyltins in mussel and liver of fish collected from certain Asian and oceanic countries. *Environmental Poll* **90:** 279-290

Kannan K, Tanabe S, Tatsukawa R (1995b) Butyltin residues in fish from Australia, Papua New Guinea and the Solomon Islands. *Intern J Environ Anal Chem* **61:** 263-273

Kannan K, Tanabe S, Tatsukawa R (1995c) Occurrence of butyltin residues in certain foodstuffs. *Bull Environ Contam Toxicol* **55:** 510-516

Kennedy W A, Griffioen W, Solmie A (1977) The 1976 crop of salmon reared on the Pacific Biological Station experimental fish farm. *Mar Fish Serv Tech Rep* 726: 21 pp

Kent M L (1992) Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. *Canadian Special Publication of Fisheries and Aquatic Sciences*, no. 116, 76 pp

Kilar J A, Mclachlan J (1986) Ecological studies of the alga *Acanthophora-specifera* Vahl Borg. Ceramiales Rhodophyta vegetative fragmentation. *J Exp Mar Biol Ecol* **104:** 1-22

King H (1995) Production of "Out of Season" smolts by photoperiod manipulation: 1995 status report. In: *Proc 1994-1995 Research and Development Review Seminar,* 17th October, 1995, pp. 33-51

Klust G, Kuhl H (1960) Tests on the prevention of marine growth on fishing equipment in coastal fisheries. *Fischerblatt* **8:** 52-56

Knoph M B, Thorud K (1996) Toxicity of Ammonia to Atlantic salmon (*Salmo salar* L.) in seawater - effects on plasma osmolarity, ion, ammonia, urea and glucose levels and hematologic parameters. *Comp Biochem Physiol* **113A:** 375-381

Koops H (1971) Prevention of marine growth on netcages in the Western Baltic Sea. *Archiv für Fischereiwissenschaft* **22**: 65-67 (in German)

Kuwa M (1984) Fouling organisms on floating cage of wire netting and the removal by *Oplegnathus* sp. cultured with other marine fish. *Bull Jap Soc Sci Fish* **50**: 1635-1640 (in Japanese)

Lagler K F, Bardach J E, Miller R R, Passino D R M (1977) *Icthyology*, 2nd edition, John Wiley & Sons, New York, 506 pp

Lai H C, Kessler A O, Khoo L E (1993) Biofouling and its possible modes of control at fish farms in Penang, Malaysia. *Asian Fisheries Science* **6**: 93-116

Lee H B, Lim L C, Cheong L (1985) Observations on the use of antifouling paint in netcage fish farming in Singapore. *Singapore J Primary Industry* **13**: 1-12

Lewis J A (1982) A guide to the principal marine fouling organisms, with particular reference to Cockburn Sound, W.A. Defence Science and Technology Organisation, Materials Research Laboratories, Australia, 27 pp

Lewis J A (1988) Organotin antifouling paints and the environment. *Surface Coatings Australia* **25**: 18-2

Lewis J A (1994a) Biofouling and fouling protection: a defence perspective. In: Kjelleberg S, Steinberg P (eds), *Biofouling: Problems and Solutions*, Proceedings of an International Workshop, The University of New South Wales, Sydney, Australia, pp 39-43

Lewis T (1993) Progress report: Biofouling research 1992/1993. In: *Proc 1993 Research and Development Review Seminar*, 29th April, 1993, pp 175-183

Lewis T (1994b) Impact of biofouling on the aquaculture industry. In: Kjelleberg S, Steinberg P (eds), *Biofouling: Problems and Solutions*, Proceedings of an International Workshop, The University of New South Wales, Sydney, Australia, pp 32-38

Lewis T (1994c) Progress report: biofouling research 1993/1994. *Proc 1993-1994 SALTAS Research and Development Review Seminar*, 18 August 1994, 219-226

Li S (1994) Fish culture in cages and pens. In: Li S, Mathias J (eds) *Freshwater Fish Culture in China: Principles and Practice*, Elsevier, Amsterdam, pp 305-346

Lindner E (1994) Low surface free energy fouling resistant coatings. In: Thompson M-F, Nagabhushanam R, Sarojini R, Fingerman M (eds) *Recent Developments in Biofouling Control*, A A Balkema, Rotterdam, pp 305-319

Littlewood D T J (1990) Polyculture of tropical bivalve molluscs with tilapia: A potentially profitable solution to predator control and fowling problems. *Aquabyte* **3**: 2

Løland G (1993a) Water flow through and around net pens. In: Fish Farming Technology (eds H Reinersten, L A Dahle, L. Jørgensen, K Tvinnereim), A A Balkema, Rotterdam, pp 177-183

Løland G (1993b) Current forces on, and water flow through and around, floating fish farms. *Aquaculture International* **1:** 72-89

Lumsden J S, Wright P, Derksen J, Byrne P J, Ferguson H W (1993) Paralysis in farmed Arctic char (*Salevlinus alpinus*) associated with ammonia toxicity. *Veterinary Record* **133:** 422-423

Mak P M S (1982) Biofouling of mariculture cages in Hong Kong. Ph.D. Thesis, University of Hong Kong, 534 pp

Maki J S, Yule A B, Rittschof D, Mitchell R (1994) The effect of bacterial films on the temporary adhesion and permanent fixation of cypris larvae, *Balanus amphitrite* Darwin. *Biofouling* **8:** 121-131

Marsland A M (1975) Ship-fouling algae, with reference to the effects of underwater scrubbing. Masters thesis, University of Newcastle Upon Tyne, 76 pp

Meadows P S, Campbell J I (1978) *An Introduction to Marine Science.* Blackie, London, 176 pp

Menton D J, Allen J H (1991) Spherical (Kiel) and square steel cages: First year of comparative evaluations at St. Andrews, NB. *8th Annual Meeting of the Aquaculture Association of Canada, Bull Aquacult Assoc Canada* **91**: 111-113

Meyers T R (1984) Marine bivalve molluscs as reservoirs of viral finfish pathogens: significance to marine and anadromous finfish culture. *Mar Fish Rev* **46:** 14-17

Mihm J W, Loeb G I (1988) The effect of microbial biofilms on organotin release by an antifouling paint. In: Houghton D R, Smith R N, Eggins H O W (eds) *Biodeterioration 7*, Elsevier Applied Science Publishing Co, London, pp 309-314

Milne A, Callow M E (1985) Non-biocidal antifouling processes. *Trans I Mar E (C)* **97:** 229-233

Milne P H (1969) Fish farm enclosures. 1. Marine growths on netting. *World Fish* **18:** 26-28

Milne P H (1970) Fish farming: A guide to the design and construction of net enclosures. *Marine Research*, No. 1, Department of Agriculture and Fisheries for Scotland, H.M.S.O., Edinburgh. 31 pp

Milne P H (1975a) Fouling of marine cages, part 1. *Fish Farm Int* 2:15-19

Milne P H (1975b) Fouling of marine cages, part 2. Further trials in Scottish lochs. *Fish Farm Int* 2:18-21

Milne P H (1979) Selection of sites and design of cages, fishpens and net enclosures for aquaculture. In: Pillay T V R, Dill W A (eds) *Advances in Aquaculture*, Papers presented at the FAO Technical Conference on Aquaculture, Kyoto, Japan, 26 May - 2 June, 1976. Fishing News Books Ltd, England, pp 416-423

Milne P H, Powell H T (1967) Preliminary report on marine fouling of fish netting test panels at Millport. *Department of Civil Engineering Report,* University of Strathclyde and S.M.B.A., 14 pp

Milne P H, Powell H T (1970) Final report on marine fouling of fish netting test panels at Millport, Loch Sween, Loch Creran, Dunstaffnage Bay. *Department of Civil Engineering Report,* University of Strathclyde and S.M.B.A., (HO-70-5), 45 pp

Moring J R (1973) Aspects of growth, and the effects of some environmental factors on pen-related Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in Puget Sound, Washington. Ph.D. Thesis, University of Washington, 225 pp

Moring J R, Moring K A (1975) Succession of net biofouling material and its role in the diet of pen-cultured Chinook salmon. *Prog Fish-Cult* **37**: 27-30

Moss B, Marsland A (1976a) The effects of underwater scrubbing on marine fouling algae. In: *Proceedings, International Marine and Shipping Congress*, pp 39-43

Moss B, Marsland A (1976b) Regeneration of *Enteromorpha. Br Phycol J* **11:** 309-311

Munday B L (1996) Infectious diseases of finfish. *Fish Health Workshop*, Proceedings 265, Post Graduate Foundation in Veterinary Science, University of Sydney, Australia, pp 81-123

Munteanu N, Maly E J (1981) The effect of current on the distribution of diatoms settling on submerged glass slides. *Hydrobiologia* **78:** 273-282

Mussalli Y G(1989) *Nontoxic Foul-Release Coatings.* Electric Power Research Institute Report No GS-6566, Project RP1689-9, Electric Power Research Institute, Palo Alto, USA

Nevell T G, Edwards D P, Davis A J, Pullin R A (1996) The surface properties of silicone elastomers exposed to seawater. *Biofouling* **10:** 199-212

Nickels J S, Parker J H, Bobbie R J, Martz R F, Lott D F, Benson P H, White D C (1981) Effect of cleaning with flow-driven brushes on the biomass and community composition of the marine microfouling film on aluminium and titanium surfaces. *Int Biodet Bull* **17:** 87-94

NSTM (Naval Ships' Technical Manual) (1989) *Waterborne Underwater Hull Cleaning of Navy Ships.* S9086-CQ-STM-010, Chapter 081, 2nd revision, 66 pp

Okamura B (1986) Formation and disruption of aggregates of *Mytilus edulis* in the fouling community of San Francisco Bay, California. *Mar Ecol Prog Ser* **30:** 275-282

Osawa Y, Tawara Y, Taketomi H (1985) Studies on behaviour of fish cages against flow of water. On relationship between volume of fish cages and current velocity. *Bulletin. Natural Research Institute for Fisheries Engineering (Japan)* **6:** 297-321 (in Japanese)

O'Sullivan D, Kiley T (1996) Status of Australian aquaculture in 1994/1995. *Austasia Aquacult*, Trade Directory, pp 2-12

Paclibare J O, Evelyn T P T, Albright L J, Prosperi-Porta L (1994) Clearing of the kidney disease bacterium *Renibacterium salmoninarum* from seawater by the blue mussel Mytilus edulis, and the status of the mussel as a reservoir of the bacterium. *Dis Aqua Org* **18**: 129-133

Pandolfini P P, Avery W H (1978) Measurement of the effect of biofouling and cleaning on the heat transfer characteristics of large diameter tubes. *Proc 5th Ocean Thermal Energy Conversion Conf*, Feb 20-22, 1978, Miami Beach, Florida, pp 41-44

Pantastico J B, Baldia J P (1981) An assessment of algal growth in net cages in Laguna Lake. *Fish Res J Philipp* **6**: 19-25

Paul J H, Jeffrey W H (1985) Evidence for separate adhesion mechanisms in hydrophilic and hydrophobic surfaces in *Vibrio proteolytica. Appl Environ Microbiol* **50:** 431-437

Pearce F (1994) Offshore petroleum. In: Kjelleberg S, Steinberg P (eds), *Biofouling: Problems and Solutions*, Proceedings of an International Workshop, The University of New South Wales, Sydney, Australia, pp 19-31

Powlik J J (1995) The effects of discarded plastic on the colonisation of nearshore substrata. *Sarsia* **80:** 229-236

Price R R, Patchan M, Clare A, Bonaventura J, Rittschof D (1991) Elimination of rosin from vinyl antifouling coatings by the use of novel cylindrical channel-forming microtubules. *Polymers in the Marine Environment,* 23-24 October, 1991, pp 145-151

Price R R, Patchan M, Clare A, Rittschof D, Bonaventura J (1992) Performance enhancement of natural antifouling compounds and their analogs through microencapsulation and controlled release. *Biofouling* **6:** 207-216

Rahav O, Ben-Zion M, Achituv Y, Dubinsky Z (1991) A photographic, computerized method for in situ growth measurements in reef-building cnidarians. *Coral Reefs* **9:** 204

Rajagopal S, Venugopalan V P, Nair K V K, Azariah J (1991) Biofouling and its control in a tropical coastal power station: a case study. *Biofouling* **3:** 325-338

Readman J W (1996) Antifouling herbicides - a threat the marine environment? *Mar Pollut Bull* **32:** 320-321

Rittschof D, Clare A S, Gerhart D J, Bonaventura J, Smith C, Hadfield M G (1992) Rapid field assessment of antifouling and foul-release coatings. *Biofouling* **6:** 181-192

Rittschof D, Sasikumar N, Murlless D, Clare A S, Gerhart D, Bonaventura J (1994) Mixture interactions of lactones and furans and a commercial biocide: toxicity and antibarnacle settlement activity. In: Thompson M F, Nagabhushanam R, Sarojini R, Fingerman M (eds) *Recent Developments in Biofouling Control*, A A Balkema, Rotterdam, pp 269-282

Robinson M G, Brown L N, Quenneville M L, Hall B D (1992) Aspects of copper tolerance and toxicity in *Amphora coffeaeformis*. *Biofouling* **5:** 261-276

Roscoe D T, Walker G (1995) Observations on the adhesion of the calcareous tube of *Pomatoceros lamarckii* and the holdfast of *Laminaria digitata*. *Biofouling* **9:** 39-50

Rothwell G N, Nash C E (1977) *Aquaculture in Tropical Oceans*, Year 01. The Oceanic Institute, Hawaii, 82 pp

Ruokolahti C (1988) Effects of fish farming on growth and chlorophyll *a* content of *Cladophora*. *Mar Pollut Bull* **19:** 166-169

Saenger P, Stephensen W, Moverley J (1979) The subtidal fouling organisms of the Calliope River and Auckland Creek, Central Queensland. *Mem Queensland Mus* **19:** 399-412

Santhanam R, Srikrishnadhas B, Natarajan P (1983) Fouling problems in cages and pens. In: *Proc Natl Seminar on Cage and Pen Culture*, Fisheries College, Tamil Nadu Agricultural University, Tuticorin. 18-19 March, 1983. Tamil Nadu Agricultural University, pp 143-147

Schoener A (1984) Replicate fouling panels and their variability. In: Costlow J D, Tipper R C (eds), *Marine Biodeterioration: An Interdisciplinary Study*, Naval Institute Press, Maryland, pp. 207-212

Schoener A, Greene C H (1980) Variability among identical fouling panels in Puget Sound, Washington, USA. *Proc 5th Int Congr Mar Corr & Foul*, May 19-23, 1980, Barcelona, pp 213-224

Schoener A, Schoener T W (1981) The dynamics of the species-area relationship in marine fouling systems: 1. Biological correlates of changes in the species-area slope. *Am Nat* **118:** 339-360

Sharma R N (1979) Cage fish culture in Nepal. *Proc IDRC/SEAFDEC Int Workshop on Cage and Pen Culture of Fish,* Tigbauan, Iloilo, Philippines, 11-22 Feb 1979, pp 93-96

Shea C, Nunley J W, Williamson J C, Smith-Sommerville H E (1991) Comparison of the adhesion properties of *Delaya marina* and the exopolysaccharide-defective mutant strain DMR. *Appl Env Microbiol* **57:** 3107-3113

Shepherd S A, Womersley H B S (1970) The sublittoral ecology of West Island, South Australia. 1. Environmental features and the algal community. *Trans Royal Society of South Aust* **94:** 105-138

Short J W, Thrower F P (1986) Accumulation of butyltin in mussel tissue of Chinook salmon reared in sea pens treated with tri-*n*-butyltin. *Mar Pollut Bull* **17:** 542-545

Silvert W (1992) Assessing environmental impacts of finfish aquaculture in marine waters. *Aquaculture* **107:** 67-79

Silvert W (1994) Simulation models of finfish farms. *J App Ichthyology* **10:** 349-352

Solbé J (1988) Water quality. In: Laird L M, Needham T (eds) *Salmon and trout farming*, John Wiley & Sons, New York, pp 69-86

Sokal R R, Rohlf F J (1981) *Biometry*. W H Freeman and Company, New York, 859 pp

Sokal R R, Rohlf F J (1995) *Biometry*, 3rd edition, W H Freeman & Company, New York

Sutterlin A M, Merrill S P (1978) Norwegian salmonid farming. *Tech Report Fisheries Marine Services of Canada*, No. 779, 47 pp

Svane I, Dolmer P (1995) Perception of light at settlement: a comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *J Exp Mar Biol Ecol* **187:** 51-61

Swain G W, Griffith J R, Bultman J D, Vincent H L (1992) The use of barnacle adhesion measurements for field evaluation of non-toxic foul release surfaces. *Biofouling* **6:** 105-114

Swain G W, Schultz M P (1996) The testing and evaluation of non-toxic antifouling coatings. *Biofouling* **10:** 187-197

Targett N M, Stochaj W R (1994) Natural antifoulants and their analogs: applying nature's defence strategies to problems of biofouling control. In: Thompson M-F, Nagabhushanam R, Sarojini R, Fingerman M (eds) *Recent Developments in Biofouling Control*, A A Balkema, Rotterdam, pp 221-227

Todd J S, Zimmerman R C, Crews P, Alberte R S (1993) The antifouling activity of natural and synthetic phenolic acid sulphate esters. *Phytochem* **34:** 401-404

Tolosa I, Readman J W, Blaevoet A, Ghilini S, Bartocci J, Horvat M (1996) Contamination of Mediterranean (Côte d' Azur) coastal waters by organotins and Irgarol 1051 used in antifouling paints. *Mar Pollut Bull* **32:** 335-341

Tomi W, Naiki K, Yamada Y (1979) Investigations into technical development of mariculture on commercial scale applied to offshore region. *Proceedings of the Japan-Soviet Joint Symposium on Aquaculture* **7:** 111-120

Tseng W Y, Yuen K H (1979) Studies on fouling organisms on mariculture nets and cages in Hong Kong. In: *Proc Aquatic Environment in Pacific Region.* Aug 21-23, 1978, Taipei, China 151-159

Vasishtha N, Sundberg D C, Rittschof D (1995) Evaluation of release rates and control of biofouling using monolithic coatings containing an isothiazolone. *Biofouling* **9:** 1-16

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar Ecol Prog Ser* **58:** 175-189

Walters L J, Smith C M (1994) Rapid rhizoid production in *Halimeda-Discoidea decaisne* (Chlorophyta, Caulerpales) fragments - a mechanism for survival after separation from adult thalli. *J Exp Mar Biol Ecol* **175:** 105-120

Wee K L (1979) Ventilation of floating cages. M.Sc. Thesis, University of Stirling, 42 pp

Weisman G R, Sundberg D C, Cimini R A, Brown M G, Beno B R, Eighmy T T (1992) Controlled release antifouling coatings. I. Approaches for controlled release of 2,4-dinitrophenolate and benzoate into seawater. *Biofouling* **6:** 123-146

Wildish D J, Keizer P D, Wilson A J, Martin J L (1993) Seasonal changes of dissolved oxygen and plant nutrients in seawater near salmonid net pens in the macrotidal Bay of Fundy. *Can J Fish Aquat Sci* **50**: 303-311

Willemsen P R, Ferrari G M (1993) The use of anti-fouling compounds from sponges in anti-fouling paints. *Surface Coatings Int* **10:** 423-427

Willinsky M D, Robson D R, Vangool W J, Fournier R A, Allen J H (1991) Design of a spherical, submersible, self-cleaning aquaculture system for exposed sites. In: Hirata G N (ed) *Proceedings of the National Science Foundation Workshop on Offshore Engineering and Mariculture*, 25-30 Sep 1991, Honolulu, USA, pp 317-336

Woods D C, Fletcher R L, Jones E B G (1988) Microfouling film composition, thickness and surface roughness on ship trial antifouling paints. In: Houghton D R, Smith R N, Eggins H O W(eds) *Biodeterioration 7*, Elsevier Applied Science Publishing Co, London, pp 49-56

Woods Hole Oceanographic Institution (1952) Marine fouling and its prevention. United States Naval Institute, Maryland, 388 pp

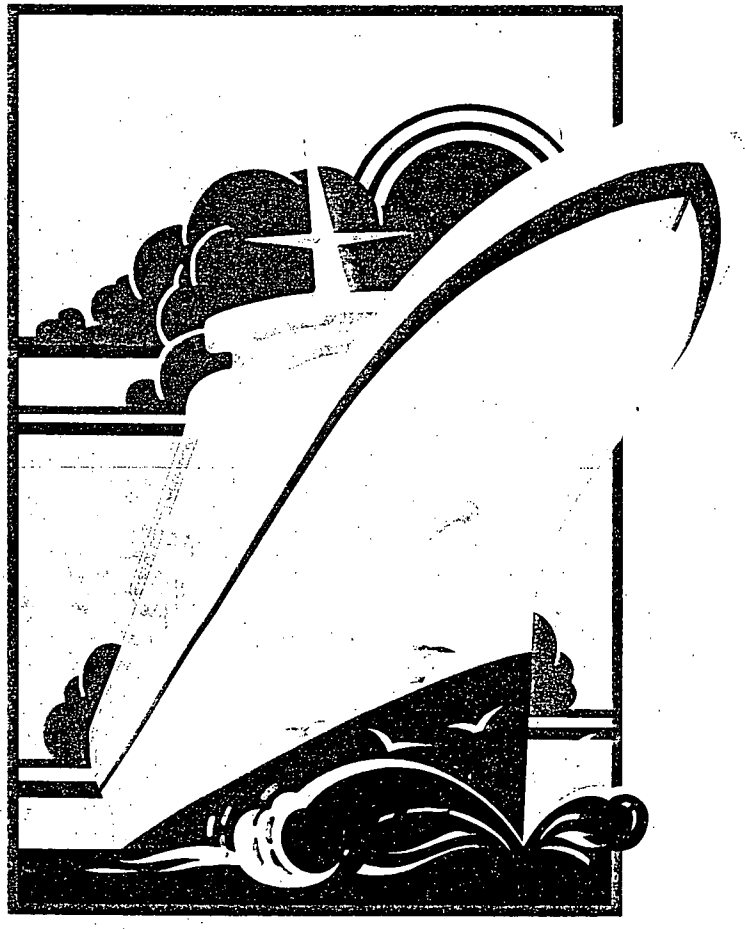
Wright M, Bakus G J, Ortiz A, Ormsby B, Barnes D M (1991) Computer image processing and automatic counting and measuring of fouling organisms. *Comp Biol Med* **21**: 173-180

Wynne K J, Tai H, Nissan R, Gardella J, Chen X (1993) *Pacific Polymer Conference Reprints* 3, 219-220

APPENDIX 1

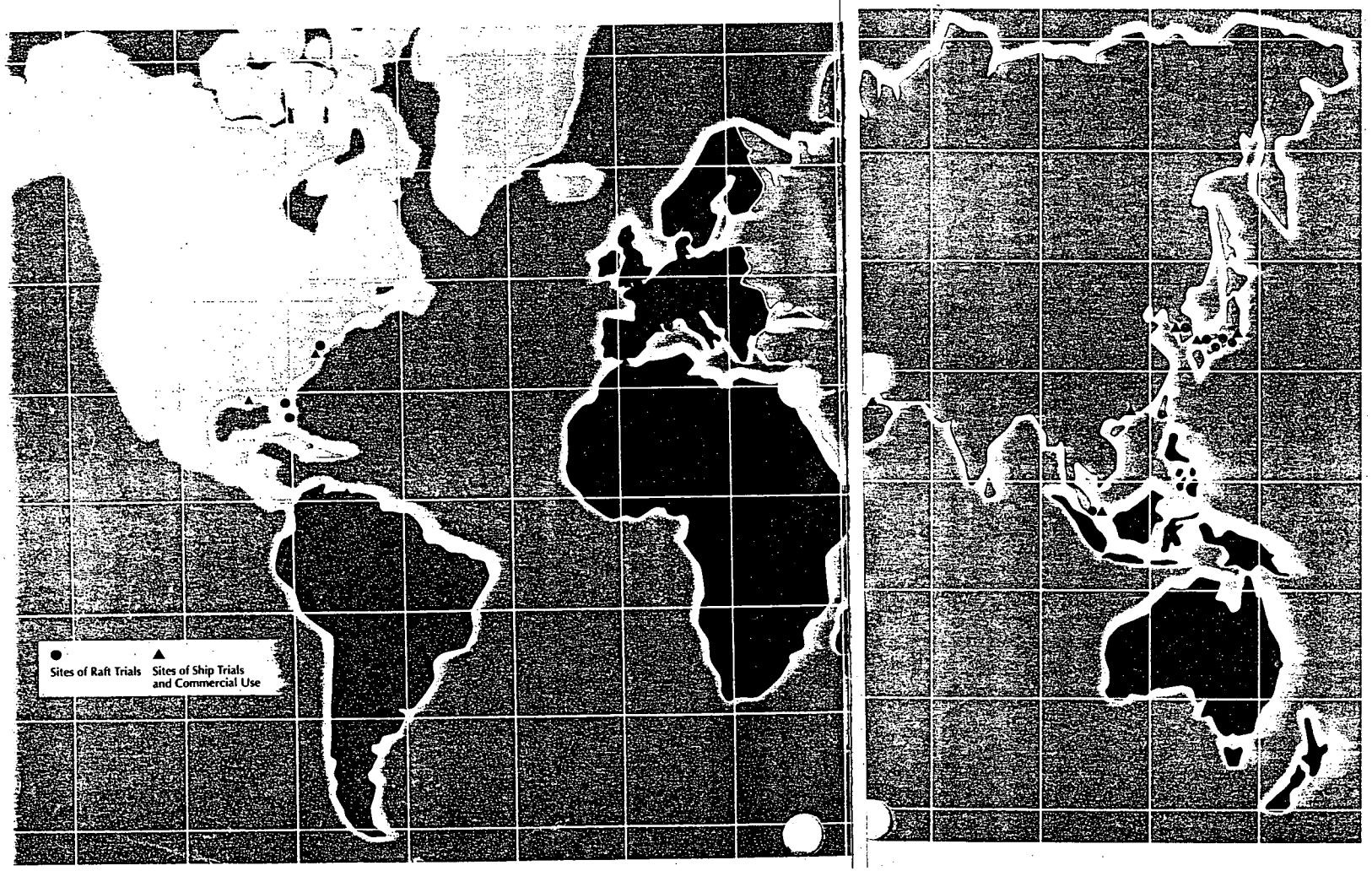
PRODUCT DATA SHEETS FOR COMMERCIALLY-AVAILABLE BIOCIDES

ROHM AND HAAS COMPANY



SEA-NINE™ 211

BIOCIDE



SEA-NINE 211 ANTIFOULING AGENT

Sea-Nine 211 antifouling agent is a unique isothiazolone product developed by Rohm and Haas Company. It is a solution of 30% active ingredient in xylene.

Sea-Nine 211 antifouling agent is highly effective against a wide range of fouling organisms and provides outstanding long-term protection when used in combination with cuprous oxide or other co-biocides.

Sea-Nine 211 antifouling agent degrades rapidly in seawater and sediment, once leached from antifouling coatings. Since it is not persistent in the environment, its use will not result in any environmental problems such as those associated with tin antifoulants.

PERFORMANCE BENEFITS

- Broad-spectrum activity: Controls bacterial slime, algae, barnacles, tubeworms, hydroids, bryozoa, tunicates, and diatoms
- Tin free
- Environmentally safe
- Provides long-lasting control
- Compatible with major antifouling formulations (self-polishing copolymers, vinyl, vinyl/rosin, chlorinated rubber, acrylics, etc.)
- Easily neutralized in case of accidental spills

SEA-NINE 211 BIOCIDE

A N T I F O U L I N G A G E N T

Table of Contents

INTRODUCTION

The ideal antifouling agent must be effective at controlling a wide range of fouling organisms while causing no adverse effects on the environment at recommended use concentrations. Sea-Nine 211 antifouling agent meets these criteria. When used in combination with cuprous oxide or other co-biocides, Sea-Nine 211 antifouling agent provides excellent protection against major fouling organisms. When released into the environment, it is degraded in a matter of hours by microorganisms in both sediment and seawater.

ROHM AND HAAS TECHNICAL SUPPORT

Rohm and Haas Sales and Technical Service personnel have more than 15 years of experience evaluating biocide performance in a variety of applications. Our staff can offer advice on optimizing biocide stability and efficacy in formulations. In addition, we can answer questions on biocide chemistry, environmental toxicity, safe storage, handling and use. For further information contact your local Rohm and Haas technical representative or the Rohm and Haas Company, Independence Mall West, Philadelphia, PA. USA 19105.

EFFICACY

Slime Producers

Laboratory tests demonstrate the inherent activity of a biocide against problem fouling organisms. General terminology includes:

Minimum Inhibitory Concentration (MIC) is the minimum concentration required to inhibit the growth of an organism.
LC_{50} is the concentration which is lethal to 50% of the test organisms.

Fouling initially occurs from bacteria, diatoms and protozoa, resulting in formation of a slime layer. A slime layer of just 1mm can cause an 80% increase in friction and a 15% loss in ship speed compared to a freshly coated surface*. Sea-Nine 211 antifouling agent is highly effective against slime-forming organisms. Lab data against common slime bacteria and diatoms is given below:

Efficacy Versus Diatoms

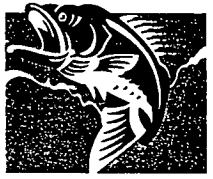
Organism	MIC (ppm ai)	UTEX No.(a)
Amphora coffeaeformis	0.4	2036
Amphipora paludosa	8	2048
Navicula incerta	0.05	2043

(a) Obtained from the Culture Collection at the University of Texas

Efficacy Versus Marine Bacteria

Organism	MIC (ppm ai)	ATCC No.
Pseudomonas atlantica	0.1	19262
Pseudomonas nautica	0.1	27132

*Bohlander, G. S., *Polymers in a Marine Environment*, October 23-24, 1991, chapter 16, page 1.



Freshwater Algae

Following the establishment of a slime layer, macrofouling rapidly develops. A number of algae contribute to the soft fouling that follows slime layer development. Sea-Nine 211 antifouling agent demonstrates excellent broad-spectrum algicidal activity.

Freshwater Algae

	MIC (ppm, ai) Sea-Nine 211	UTEX No.[a]
Green Algae		
Chlorella pyrenoidosa	0.06	1230
Chlorococcum oleofaciens	1.0	105
Scenedesmus quadricauda	1.3	614
Ulothrix acuminata	0.6	739
Blue Green Algae		
Anabaena flos-aquae	0.3	1444
Synechococcus leopoliensis	0.6	625
Nostoc commune	0.6	584
Scytonema hofmanni	0.6	2349
Microcystis aeruginosa	0.6	2063
Oscillatoria prolifera	0.3	1270

[a]Obtained from the Culture Collection of Algae at the University of Texas

Efficacy Against Barnacles

Organism	LC_{50} (ppm, ai)
Balanus amphitrite	0.34

Marine Algae

The most common fouling algae on ships are species of the green algae *Enteromorpha*. They are commonly found to be the dominant algae on copper-containing antifouling paints. The predominance of *Enteromorpha* results from its widespread distribution, enormous reproductive capacity, and ability to withstand extremes in environmental conditions.

Sea-Nine 211 antifouling agent has outstanding activity against *Enteromorpha intestinalis* and *Ectocarpus siliculosis*.

Efficacy Versus Marine Algae in a Spore Germination Test

Algae	MIC (ppm, ai)
Enteromorpha intestinalis	0.1
Ectocarpus siliculosis	0.2

Barnacles

Barnacles are the most commonly encountered hard-fouling organism. Several species are found throughout the world. Sea-Nine 211 antifouling agent, unlike most non-metallic biocides, has good activity against barnacles. When properly formulated, Sea-Nine 211 antifouling agent can provide additional efficacy against barnacles.

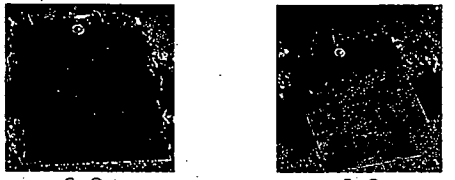
Raft Exposures

The outstanding activity of Sea-Nine 211 antifouling agent against major fouling organisms in laboratory tests was confirmed in several raft exposure tests. Tests have been conducted at numerous locations in Europe, Asia, and North America. These tests confirm the excellent activity of Sea-Nine 211 antifouling agent especially against soft-fouling organisms. Testing in different regions demonstrates efficacy against the wide array of fouling organisms found in various parts of the world. Sites of raft exposure tests are shown on the world map on page 1.

Representative results from raft exposure tests are shown below.

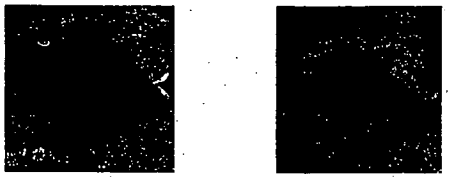
Raft Exposure Tests Conducted in New Jersey, U.S.A. (7 months exposure)

Navy 121 Paint



Cu_2O + 3% Sea-Nine 211 Biocide | Cu_2O

Vinyl Resin Paint



Cu_2O + 3% Sea-Nine 211 Biocide | Cu_2O

Vinyl/Rosin Paint

Cu_2O + 3% Sea-Nine 211 Biocide | Cu_2O

3% Sea-Nine 211 Biocide as active ingredient. See formulations on page 9.

Ship Painting Trials

The final proof of any antifouling biocide is performance on in-service vessels. The locations of these trials and commercial uses are shown on the world map on page 1. Excellent performance against the whole range of fouling organisms is seen when Sea-Nine 211 antifouling agent is used in combination with cuprous oxide or other co-biocides. Sea-Nine 211 antifouling agent has been shown to be successful in extensive worldwide ship trials and commercial use.

Environmental Fate

A marine antifouling agent must be effective at controlling a wide range of fouling organisms without causing any adverse effects on the environment. The ideal antifouling agent should: 1) rapidly degrade in the environment, 2) not bioaccumulate, and 3) show no chronic toxicological effects at sublethal doses. Sea-Nine 211 antifouling agent meets all of these critria. When released into the environment, Sea-Nine 211 antifouling agent is degraded within hours by microorganisms. No chronic effects have been observed in any species tested, and bioaccumulation is < 1 percent. A comparison of the environmental fate of TBTO (tributyl tin oxide) and Sea-Nine 211 antifouling agent is shown on pages 6, 7, 8 and 9.

Since organotin biocides are the industry standard, we have shown data on TBTO taken from the literature for comparison.

Environmental Degradation

Biocides can be degraded in the environment by hydrolysis, UV light, biodegradation, and other routes. Degradation can occur in both seawater and the sediment. Degradation is often characterized by a half-life, the time required for half of the initial biocide concentration to be degraded.

Sea-Nine 211 antifouling agent	TBTO
• Half-life in seawater is < 24 hours. • Half-life in sediment is < 1 hour. • The primary mechanism of degradation is biological.	• Half-life ranging from weeks to several months in seawater. • Half-life in sediment varies from 6 to 9 months. • Degradation occurs primarily by biodegradation, however, monitored levels of TBTO in field studies are at concentrations that are lethal to the metabolizing microorganisms.

Environmental Partitioning

When a biocide is released into the environment, it partitions between seawater and the sediment. Biocides that bind tightly to the sediment are not bioavailable to adversely affect marine organisms.

Sea-Nine 211 antifouling agent	TBTO
• Sea-Nine 211 antifouling agent and its degradation products rapidly partition into sediment and remain essentially immobile. This results in limited bioavailability and a reduced potential for toxicity.	• Partitions approximately 95% into the water and is bioavailable. • Sediment can serve as a sink for continual renewal of TBTO into the water. • Desorption from sediment is facilitated by agitation.

Aquatic Toxicology

High concentrations of antifoulant biocides are toxic to marine organisms. The key factors affecting their environmental impact are:
1) how rapidly they are metabolized;
2) how toxic their metabolites are; and
3) chronic toxic effects observed at sublethal concentrations.

Sea-Nine 211 antifouling agent	TBTO
• Acutely toxic at low ppb levels. • Rapidly degraded to less toxic metabolites. • No reproductive or chronic toxicological effects are observed in marine organisms.	• Acutely toxic at low ppb levels. • Degradation occurs to dibutyltin species which are still toxic and highly persistent. • Dramatic effects on growth, development, and reproduction are observed at low part per trillion levels. • Oyster malformations have been observed at 7 to 14 parts per trillion. • Imposex in gastropods can occur at concentrations of 10 to 20 parts per trillion.

SEA-NINE 211
BIOCIDE

Bioaccumulation

When a biocide is released into seawater, it enters the food chain for marine organisms. If it is not metabolized, the biocide may be stored in that organism and bioaccumulate to levels substantially higher than those found in the environment.

Sea-Nine 211 antifouling agent	TBTO
• Rapidly metabolized and incorporated into natural products. • Bioaccumulation is nil.	• Undergoes significant bioaccumulation. • In some bivalves, bioaccumulation can result in the TBTO concentration in the bivalve tissue being 10,000 times higher than the concentration found in the environment.

Maximum Acceptable Envirnmental Concentration

Based on the susceptibility of the most sensitive marine organism, an MAEC (maximum acceptable environment concentration) can be established.

Sea-Nine 211 antifouling agent	TBTO
MAEC = 0.63 ppb	MAEC = 0.001 ppb (UK Environmental Quality Target set at 0.002 ppb)

Environmental Concentrations

Using environmental fate models in which worst-case assumptions are made, the environmental concentration of a biocide can be predicted. Monitored concenrations of tin biocides agree closely with those concentrations predicted by these models, demonstrating their validity for risk assessment.

Sea-Nine 211 antifouling agent	TBTO
• Would have a concentration of approximately 1 to 10 parts per trillion assuming total usage of this biocide on all ships. • Predicted worst-case concentrations are 1.5 to 2.5 orders of magnitude less than the MAEC.	• Concentrations have been monitored throughout the U.S. of 0.01 to 1ppb. These monitored concentrations agree closely with those predicted by environmental models and are 2 to 3 orders of magnitude greater than the MAEC. • Levels of 10 to 20 parts per trillion have recently been measured in the North Sea as far as 200 km off shore.* *Coghlan, A., *New Scientist*, December 8, 1990, pg 16.

FORMULATION GUIDELINES

Sea-Nine 211 antifouling agent is compatible with common marine antifoulant formulations such as self-polishing copolymers, vinyl, chlorinated rubber, vinyl/rosin, acrylic/rosin, alkyd/rosin, and oleoresinous paints. To obtain the best performance, optimization of leach rates is required. Typical formulations are listed below and at right.

Soluble Matrix Coating
(based on Navy Military Spec #121)

Ingredients	Weight %
Sea-Nine 211 antifouling agent	9.3
Gum Rosin	10.5
Vinyl Resin	2.7
Plasticizer	2.4
MIBK	7.2
Cuprous Oxide	67.5
Antisettling Agent	0.4

Vinyl/Rosin Coating

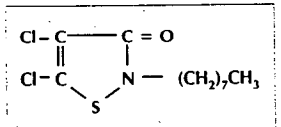
Ingredients	Weight %
Sea-Nine 211 antifouling agent	9.0
Plasticizer	2.0
Gum Rosin	5.0
Vinyl Resin	15.4
Xylene	19.8
MIBK	22.8
Cuprous Oxide	25.0

Chlorinated Rubber Coating

Ingredients	Weight %
Sea-Nine 211 antifouling agent	9.5
Chlorinated Rubber Binder	10.6
Gum Rosin	21.2
Plasticizer	2.0
Xylene	30.6
Cuprous Oxide	24.7
Antisettling Agent	1.4

PHYSICAL AND CHEMICAL PROPERTIES

Sea-Nine 211 antifouling agent is a patented formulation containing a 30% solution of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one in xylene.



CAS Registration No. 64359-81-5
Molecular Formula: $C_{11}H_{17}Cl_2NOS$
Molecular Weight: 282.2

Determination of active ingredient quantity can be performed by HPLC or gas chromatography. Detailed methods are available on request.

The physical and chemical characteristics of the technical-grade active ingredient are:

Typical Physical Properties	
Color	Tan to brown
Melting point	40–41°C
Density	1.28 g/ml at 25°C
Vapor pressure	$7.4X10^{-6}$ torr at 25°C
Octanol/water partition coefficient (log P)	6.4
Solubility (25°C)	
Water	6 ppm
Acetone	~65%
Ethanol	~65%
Cellosolve	~30%
Xylene	~65%
MIBK	~60%
Solvent Naphtha	~25%

Product Stability: Sea-Nine 211 antifouling agent is stable for at least one year at 25°C and 40°C and at least six months at 55°C.

SAFE HANDLING INFORMATION

Sea-Nine 211 antifouling agent is moderately toxic and is not genotoxic or teratogenic.

Sea-Nine 211 antifouling agent, like organotin biocides, is corrosive to the eyes and can be a skin sensitizer. For a detailed toxicity profile on Sea-Nine 211 antifouling agent consult Rohm and Haas publication CS-621 **Toxicity of Sea-Nine 211 Antifouling Agent.**

Sea-Nine 211 antifouling agent is safe to use if proper industrial hygiene procedures, which prevent inhalation of vapors or mists and skin and eye contact, are followed. Recommended procedures are described in Rohm and Haas publication CS-622, **Safe Handling of Sea-Nine 211 Antifouling Agent.**

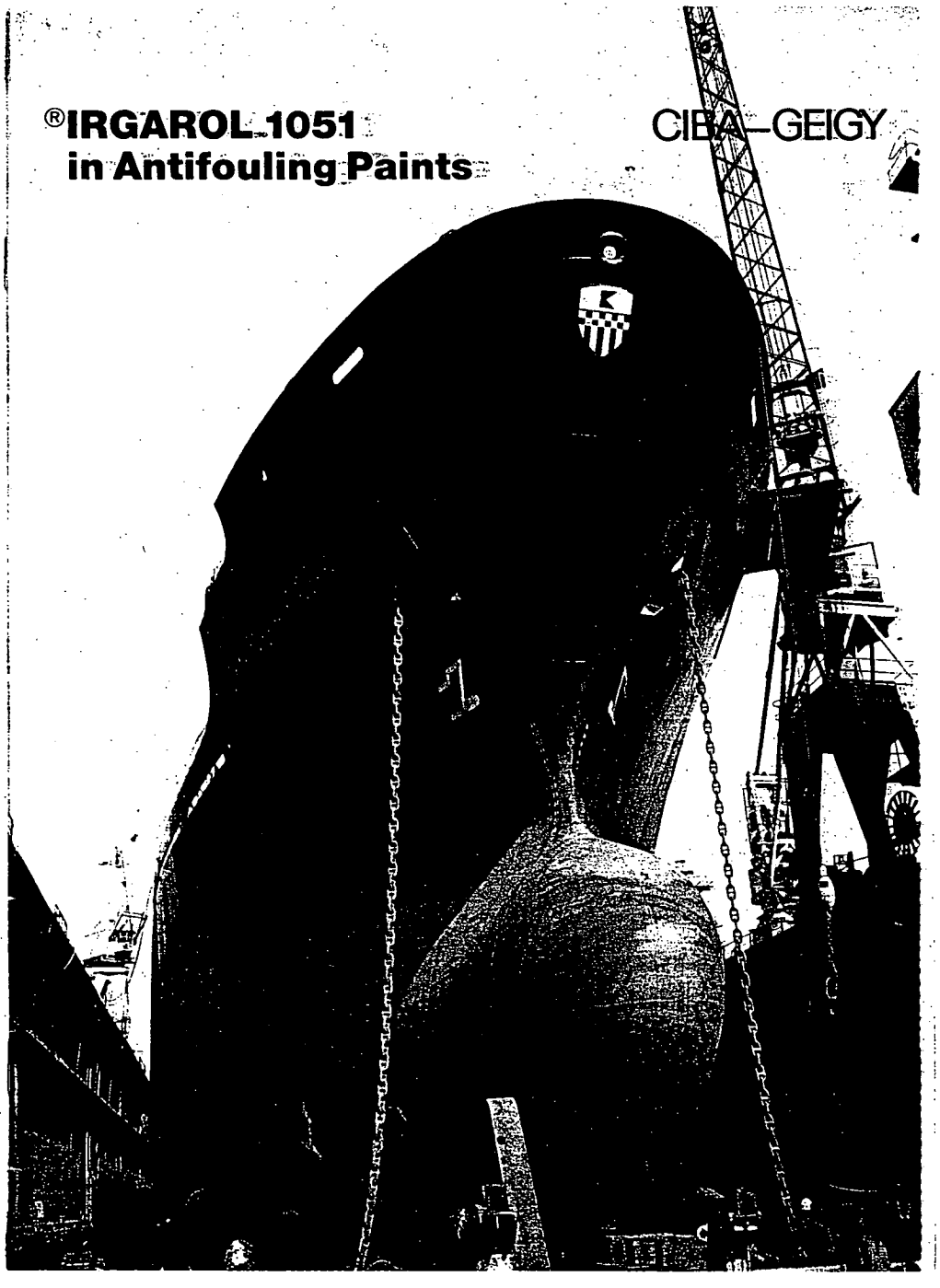
MATERIAL SAFETY DATA SHEETS

Material Safety Data Sheets (MSDS) are available for all Rohm and Haas products. These sheets contain pertinent information that you may need to protect your employees and customers against any known health or safety hazards associated with our products.

We recommend that you obtain copies of our MSDS from your local Rohm and Haas technical representative before using our products in your facilities. We also suggest that you contact your suppliers of other materials recommended for use with our products for appropriate health and safety precautions before using them.

®IRGAROL 1051 in Antifouling Paints

CIBA–GEIGY



200

Contents

Laboratory tests

The excellent effectiveness of IRGAROL 1051 against algae can be demonstrated in laboratory tests.

The minimal inhibition concentration (MIC) is the concentration required to inhibit the growth of a specific alga.

The minimal killing concentration (MKC) is the concentration required to impair the alga to such an extent that it cannot recover in algicide-free water.

Freshwater algae

Minimal inhibition concentration against freshwater algae

	MIC mg/l IRGAROL 1051
Blue algae	
Oscillatoria geminata	1
Nostoc spec.	0.1
Phormidium foveolarum	0.1
Anacystis nidulans	0.1
Scytonema stuposum	0.1
Green algae	
Chlorella vulgaris	0.1
Chlorella pyrenoidosa	0.1
Scenedesmus spec.	0.1
Ulothrix subtilissima	0.1
Type of brown alga	
Tribonema aequale	0.1

Testing in algae medium

In practice algae grow on the protective surface. In the agar incorporation test, the substance to be tested is located in the agar and the algae grow on the surface. Therefore a comparison of the activities of various algae controlagents in the agar incorporation test provides particularly effective simulation of practical conditions.

	MIC mg/l				
Type of alga	IRGAROL 1051	TBTO	TBTF	TPTC	TPLA
Oscillatoria geminata	0.03	0.3	3	0.3	0.3
Nostoc. spec.	0.03	0.3	0.3	0.3	0.3
Phormidium foveolarum	0.03	1	0.3	0.3	1
Anacystis nidulans	0.03	0.3	0.3	0.3	0.3
Chlorella vulgaris	0.3	10	3	3	10
Chlorella pyrenoidosa	0.03	0.3	0.3	1	10
Scenedesmus spec.	0.03	1	1	0.3	3
Ulothrix subtil.	0.3	1	1	0.3	1
Tribonema aequlae	0.03	0.3	0.3	0.3	1

TBTO – bistributyltin oxide
TBTF – tributyltin fluoride
TPTC – triphenyltin chloride
TPLA – triphenyllead acetate

Testing on algae agar

This comparison clearly shows the superiority of IRGAROL 1051 with respect to its inhibiting effect against freshwater algae, the lower the value, the higher the effectiveness.

Seawater algae

Determination of effectiveness of IRGAROL 1051 against seawater algae

An investigation of algae on oil tankers lying in European harbours revealed the following distribution:

75% enteromorpha
13% ectocarpus
5% cladophora
5% chaetomorpha
2% other algae

For this reason the effectiveness against the green alga, enteromorpha intestinalis, which is the most important in sea water fouling, was tested in sterile-filtered sea water containing soil extract. To determine the minimal inhibition concentration (MIC) the alga remains in the sea water containing the active substance for 8 weeks and then, after this incubation at 18 °C, the growth is evaluated.

The activity of IRGAROL 1051 against the brown alga ectocarpus intestinalis and the green alga cladophora was tested in a similar fashion.

An algae control agent which inhibits growth might be effective under static conditions but if ships are moving the concentration coefficient between the paint film and sea water is largely reduced. Therefore, algae already attached will continue to grow.

Tankers lie in harbours for 5–8 hours but the time which the enteromorpha requires for attachment is only 3–5 hours. An algicide which kills off algae within this period will therefore protect antifouling paints against algal growth.

The minimal killing concentration (MKC) is determined as follows: After the incubation period the algae are taken from the sea water containing a defined amount of algicide. Then they are washed and after another incubation of 8 weeks in fresh sea water they are evaluated with respect to growth (Fig. 1) and no growth (Fig. 1a) (killing) respectively.

Fig. 1

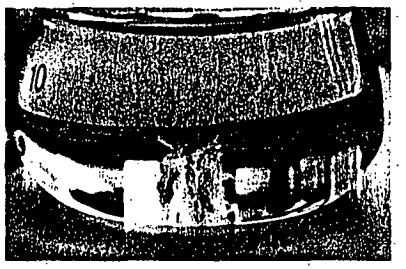


Fig. 1a

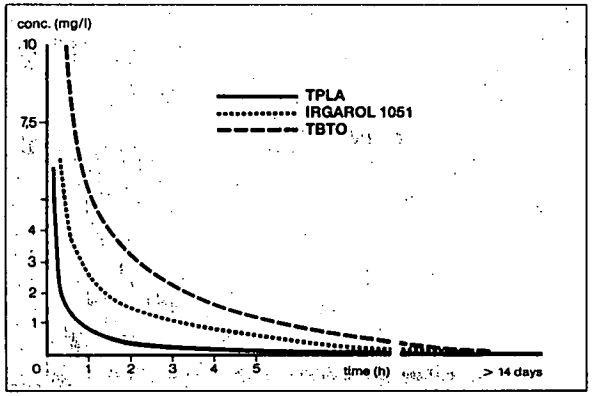
In the following table the results MKC_4 against Enteromorpha and MIC against Enteromorpha, Ectocarpus and Cladophora using IRGAROL 1051 are compared with some other algae control agents.

Product	Ectoc. silic. MIC	Cladophora MIC	Enteromorpha intestinalis MIC	Enteromorpha intestinalis MKC_4
IRGAROL 1051	0.05	0.1	0.01	0.5
TBTO	0.5	–	0.1	2
TBTF	–	–	0.1	2–5
TPTC	–	0.1	0.05	1–2
TPLA	0.5	–	0.01	0.25

Testing of sea water algae (in mg/l)

The MKC_4 is the concentration of active substance which, after 4 hours, is sufficient to affect the alga such that it cannot recover in fresh sea water.

The minimum required incubation time for killing Enteromorpha intestinalis as a function of the concentration of active substance is shown in the graph:

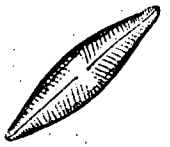


At very low concentrations IRGAROL 1051 not only inhibits the growth of Enteromorpha but within a short time also exerts such an influence on the alga that it cannot recover in fresh water.

The outstanding effectivity of IRGAROL 1051 against the brown algae and green algae was confirmed. A concentration of 0.05 ppm of IRGAROL 1051 is sufficient to inhibit the growth of Ectocarpus siliculosus as well as Cladophora sp.

Diatoms **IRGAROL 1051 as active substance against diatoms**

Primary slime on paints of ships' bottoms mainly consists of diatoms. A concentration of less than 1 ppm of IRGAROL 1051 inhibits the growth of the three bacillariophyceae tested in special sea water nutrients:



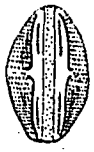
navicula pelliculosa

gomphonema parvulum

cyclotella cryptia.

IRGAROL 1051 is also effective in a mixed culture of diatoms associated with enteromorpha at a concentration of less than 1 ppm.

Practical trials have proved the outstanding effect of IRGAROL 1051 against the wide-spread diatom



amphora.

The minimal inhibition concentration of IRGAROL 1051 against different diatoms are:

strain	MIC (mg/l)
Navicula sp.	0.01
Nitzschia sp.	0.01
Amphora sp.	0.01
Achnanthes sp.	0.01
Sinnularia sp.	0.03

Raft exposure

The excellent laboratory results obtained with IRGAROL 1051 against the sea water algae enteromorpha intestinalis and ectocarpus silicolosus were confirmed by exposure of test panels in the Mediterranean Sea and in the North Sea.

These tests clearly showed that IRGAROL 1051 with organotin compounds and/or inorganic copper compounds, which are particularly effective in antifouling paints against animal organisms, provide excellent effectiveness against plant organisms. This was confirmed in trials of 20 and 32 months. In particular the combination of IRGAROL 1051 with cuprous oxide and triphenyltin or tributyltin-fluoride prevented the attachment of plant and animal organisms on paint systems.

Fig. 2.
Marine growth on test frame for antifouling paints (Mediterranean)

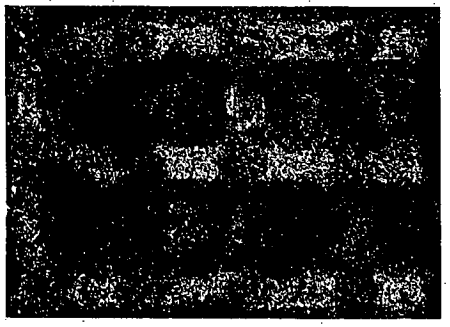
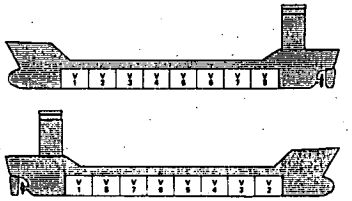


Fig. 2 a.
Antifouling paint containing IRGAROL 1051 combined with organotin and copper after 20 months immersion (Mediterranean)

Practical tests

On a 8,000 tons trading vessel test paints were applied on areas of 4 m width each. The test areas were on both sides of the vessel, i. e. reaching from the starboard and port at the height of the charging marks to the flat bottom via the drift keel (Fig. 3 to 6).



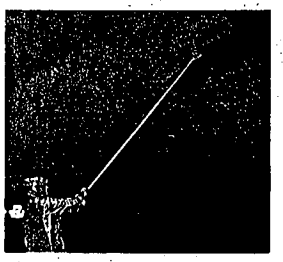


Fig. 3. Blasting before repainting.

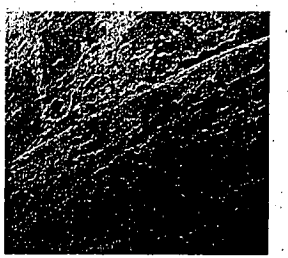


Fig. 4. The corrosive attack at the water-line area was covered with a zinc primer.

Fig. 5. After applying the chlorinated rubber primer.

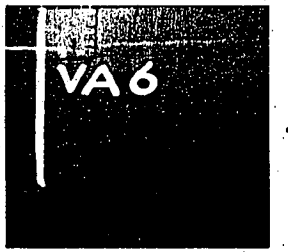
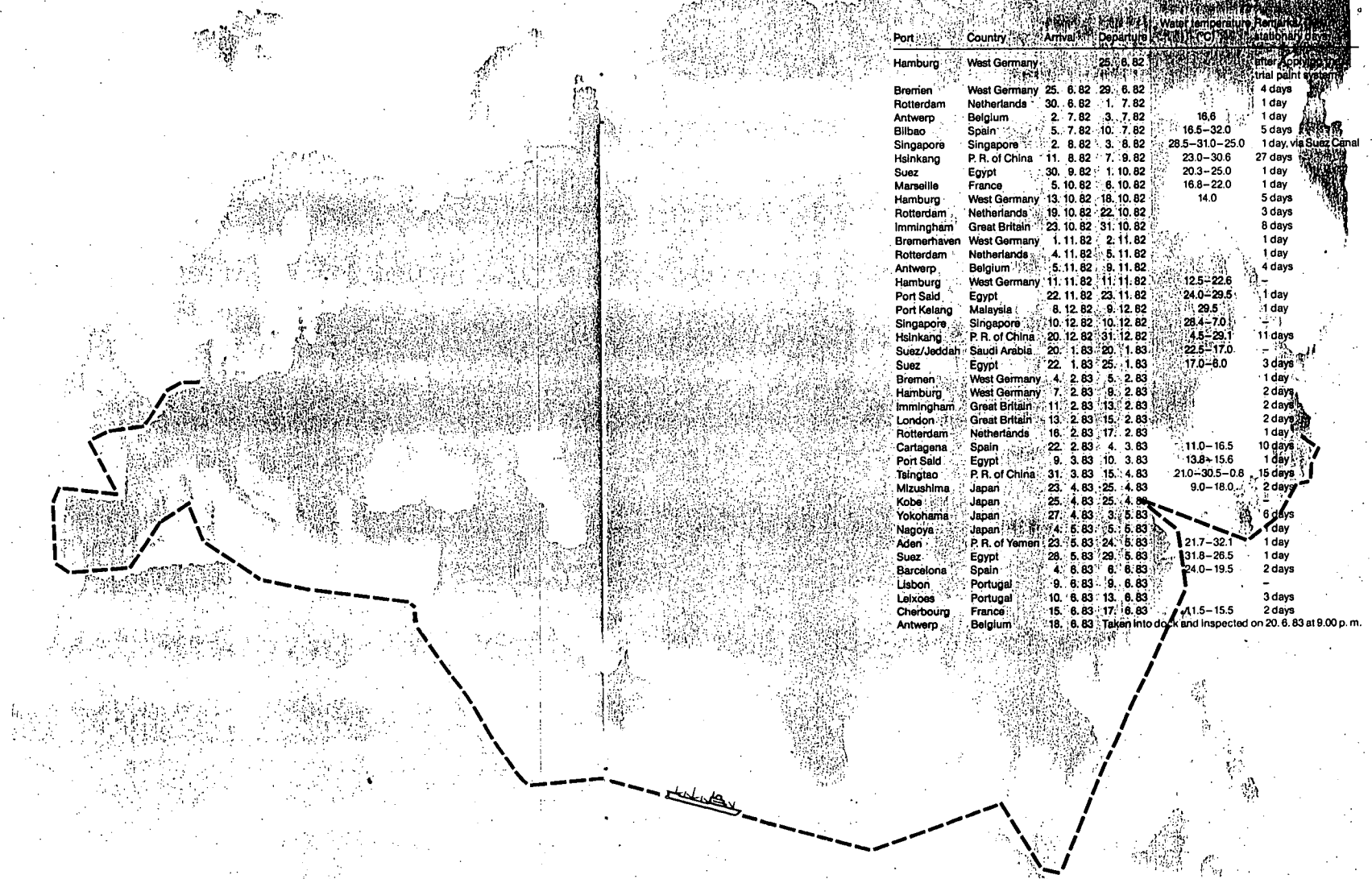


Fig. 6. Marking of the antifouling paints below the drift keel.

The trading route of the vessel included Gulf and Far Eastern ports when it spent up to 26 days at a time in port waters which are known to give rise to severe growth of algae.



Trading route and lay days of the vessel with the trial paint systems

Port	Country	Arrival	Departure	Water temperature (°C)	Remarks	Stationary days
Hamburg	West Germany		25. 6. 82			Start of the trial paint systems
Bremen	West Germany	25. 6. 82	29. 6. 82			4 days
Rotterdam	Netherlands	30. 6. 82	1. 7. 82			1 day
Antwerp	Belgium	2. 7. 82	3. 7. 82	16.6		1 day
Bilbao	Spain	5. 7. 82	10. 7. 82	16.5–32.0		5 days
Singapore	Singapore	2. 8. 82	3. 8. 82	28.5–31.0–25.0		1 day, via Suez Canal
Hsinkang	P. R. of China	11. 8. 82	7. 9. 82	23.0–30.6		27 days
Suez	Egypt	30. 9. 82	1. 10. 82	20.3–25.0		1 day
Marseille	France	5. 10. 82	6. 10. 82	16.8–22.0		1 day
Hamburg	West Germany	13. 10. 82	18. 10. 82	14.0		5 days
Rotterdam	Netherlands	19. 10. 82	22. 10. 82			3 days
Immingham	Great Britain	23. 10. 82	31. 10. 82			8 days
Bremerhaven	West Germany	1. 11. 82	2. 11. 82			1 day
Rotterdam	Netherlands	4. 11. 82	5. 11. 82			1 day
Antwerp	Belgium	5. 11. 82	9. 11. 82			4 days
Hamburg	West Germany	11. 11. 82	11. 11. 82	12.5–22.6		–
Port Said	Egypt	22. 11. 82	23. 11. 82	24.0–29.5		1 day
Port Kelang	Malaysia	8. 12. 82	9. 12. 82	29.5		1 day
Singapore	Singapore	10. 12. 82	10. 12. 82	28.4–7.0		–
Hsinkang	P. R. of China	20. 12. 82	31. 12. 82	4.5–29.1		11 days
Suez/Jeddah	Saudi Arabia	20. 1. 83	20. 1. 83	22.5–17.0		–
Suez	Egypt	22. 1. 83	25. 1. 83	17.0–6.0		3 days
Bremen	West Germany	4. 2. 83	5. 2. 83			1 day
Hamburg	West Germany	7. 2. 83	9. 2. 83			2 days
Immingham	Great Britain	11. 2. 83	13. 2. 83			2 days
London	Great Britain	13. 2. 83	15. 2. 83			2 days
Rotterdam	Netherlands	16. 2. 83	17. 2. 83			1 day
Cartagena	Spain	22. 2. 83	4. 3. 83	11.0–16.5		10 days
Port Said	Egypt	9. 3. 83	10. 3. 83	13.8–15.6		1 day
Tsingtao	P. R. of China	31. 3. 83	15. 4. 83	21.0–30.5–0.8		15 days
Mizushima	Japan	23. 4. 83	25. 4. 83	9.0–18.0		2 days
Kobe	Japan	25. 4. 83	25. 4. 83			–
Yokohama	Japan	27. 4. 83	3. 5. 83			6 days
Nagoya	Japan	4. 5. 83	5. 5. 83			1 day
Aden	P. R. of Yemen	23. 5. 83	24. 5. 83	21.7–32.1		1 day
Suez	Egypt	28. 5. 83	29. 5. 83	31.8–26.5		1 day
Barcelona	Spain	4. 6. 83	6. 6. 83	24.0–19.5		2 days
Lisbon	Portugal	9. 6. 83	9. 6. 83			–
Leixoes	Portugal	10. 6. 83	13. 6. 83			3 days
Cherbourg	France	15. 6. 83	17. 6. 83	11.5–15.5		2 days
Antwerp	Belgium	18. 6. 83	Taken into dock and inspected on 20. 6. 83 at 9.00 p. m.			

After a one year's running period the vessel was taken into dock and inspected. A commercial antifouling paint functioning as comparative paint clearly showed growth of algae at the waterline area and barnacles reaching to the flat bottom (Fig. 7).

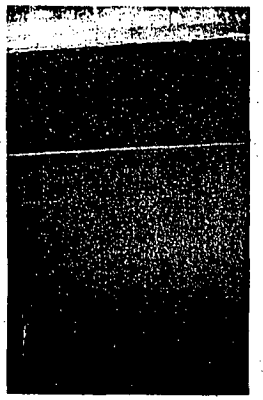


Fig. 7

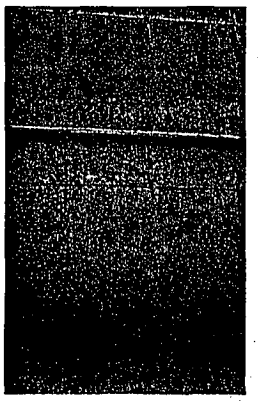


Fig. 8

The formulation containing IRGAROL 1051 in combination with tributyltin fluoride and cuprous oxide gave a result completely free of growth. Only the scrub spots on the side bulb were covered with algae (Fig. 8). A comparative formulation containing a higher amount of tributyltin fluoride and cuprous oxide, however, **without** IRGAROL 1051, showed no barnacle attachment but growth of algae (Fig. 9). In another test paint IRGAROL 1051 was added to this formulation and the pronounced attachment of algae could no longer be ascertained (Fig. 10).

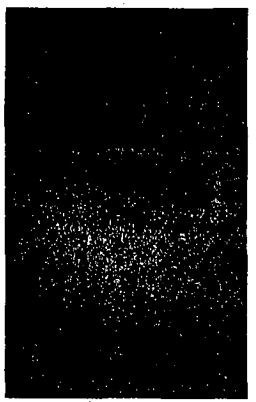


Fig. 9

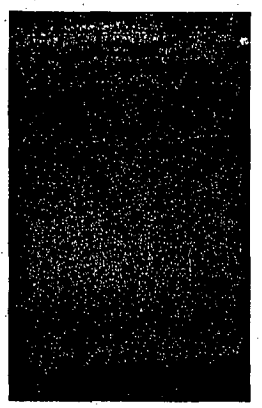


Fig. 10

	Fig. 8		Fig. 9		Fig. 10	
	wt.	vol.	wt.	vol.	wt.	vol.
®Vinylite VAGH	81.0	60.0	45.5	33.7	43.0	31.8
Cumarone resin	27.0	24.5	45.5	41.1	43.0	39.1
®REOFOS 65	4.2	4.2	11.9	11.9	11.2	11.2
®IRGAROL SA 39	13.2	13.2	4.0	4.0	9.7	9.7
Red Iron oxide 222	109.2	22.8	123.2	25.7	116.0	24.2
Baryte	100.8	22.9	112.9	25.7	106.3	24.2
Talcum	63.8	22.7	71.8	25.6	67.7	24.2
MIBK	150.0		130.3		122.3	
®Solvent naphtha	161.0					
Xylene			139.0		130.9	
IRGAROL 1051	37.2	33.8			58.5	53.2
TBTF	58.4	50.8	79.0	68.7	74.4	64.7
Cuprous oxide	194.4	33.5	236.9	40.8	223.0	38.4
	1000.0	288.4	1000.0	277.2	1000.0	314.7

Formulations of the test paints.

Application

Fields of application

The excellent effectiveness of IRGAROL 1051 as approved in the aforementioned ship trial is by far not restricted to paint formulations as described above. Careful selection of the ingredients and compositions allow the preparation of highly specialized paints and varnishes.

As already mentioned IRGAROL 1051 has no activity against attachment of animals like barnacles, serpulides, mulluscs etc. Its outstanding activity against algae is used in classical antifouling paints where it is combined with copper, cuprous oxide, organotin compounds or other suitable products active against animal settlement.

Metal free antifouling paints for regions where no animal settlement exists are protected from weed growth by incorporating IRGAROL 1051.

The effectivity of self-polishing antifoulings is based on a continuous hydrolysis of the active moiety and abrasion of the paint surface which is thus flattened. If the ships equipped with self-polishing paints are not moving, abrasion does not take place and primary slime mainly consisting of diatoms, can become attached. To avoid this IRGAROL 1051 is incorporated in the preparation of these paints, taking advantage of its high effectivity against diatoms.

In fishing net varnishes IRGAROL 1051 prevents the attachment of algae. It depends on the actual situation whether the product is used alone or in combination with other active ingredients.

The good thermostability of IRGAROL 1051 allows its use as an algicide in materials like rubber or certain synthetics as well.

For applications in emulsion paints see separate brochure.

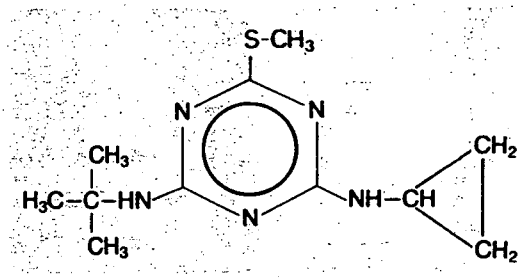
Incorporation

In most cases IRGAROL 1051 is incorporated by using the solid product. Sometimes a previously prepared paste or a solution is recommended. Large amounts of IRGAROL 1051 which have been dissolved by heating in the solvents may crystallize when cooling down. This too has to be taken into account when testing the storage stability in accelerated tests (paint control by storage at elevated temperature).

Especially in paints with a low content of pigments or/and fillers the selection of binder or the composition of binders has to be optimized with respect to the solubility of IRGAROL 1051 in the paint and its availability on the surface.

Technical data

IRGAROL 1051 is an s-Triazine of the Formula:



2-methylthio-4-tert.-butylamino-6-cyclopropylamino-s-triazine

IRGAROL 1051 is a yellowish solid substance with a melting point of about 130 °C.

Analytical characterisation and determination of IRGAROL 1051 can be performed by IR-spectrum, UV-spectrum, thin layer chromatography or gaschromatography. Detailed discription of the methods on request.

Solubility	g/l
Xylene	ca. 50
®Shellsol-A	ca. 30
Solvent naphtha	ca. 50
White spirit	ca. 10
Methylisobutylketone	ca. 120
Butylacetate	ca. 150
Ethylglycolmonomethylether	ca. 100
Butylene glycol	ca. 150
Propylene glycol	ca. 10
Octanol	ca. 50
®Texanol	ca. 50

Sulubility in organic solvents at room temperature.

The solubility as a function of the temperature for three different solvents is:

	Solubility (g/l) in		
	Xylene	MIBK	Diethyleneglycolmonobutylether
20 °C	45	90	135
40 °C	100	180	200
60 °C	240	310	340

IRGAROL 1051 has an extremely low water solubility:

Water at 20 °C pH 7 7 mg/l

Toxicological data

see separate Safety Data Sheet

Henkel

Plastics & Coatings Technology

Henkel Australia Pty Limited
ACN: 001 302 996
83 Maffra St. Broadmeadows Vic. 3047
PH: (03) 309 5188 FAX: (03) 309 9492
Sydney (Orders): (02) 748 4222

NOPCOCIDE N-96

Technical Microbicide for Manufacturing use
to Control Mildew on Paint Film.

NOPCOCIDE N-96 is a broad spectrum microbicide for control of fungi. Its high degree of activity provides excellent performance characteristics for use as a mildewcide in:-

- latex exterior and interior emulsion paints. For exterior paints, use 5 - 10 kg per 1,000 litres. For interior paints, use 2.5 - 5 kg per 1000 litres.
- solvent-based paints at levels of 5 - 12 kg per 1,000 litres.

NOPCOCIDE N-96 has several desirable properties which are important in manufacturing fungus resistant paint:-

- extremely low water solubility
- low vapour pressure
- fine particle size
- excellent pH stability.

RECOMMENDATIONS FOR USING NOPCOCIDE N-96

Latex Emulsion Paints

NOPCOCIDE N-96, a micromilled powder, is best dispersed during the pigment grinding operation. Grinding equipment normally used in the paint industry is entirely satisfactory.

Generally, 5 - 10 kg of **NOPCOCIDE N-96** per 1,000 litres of exterior paint are sufficient for effective mildew control in the paint film. In extreme mildew areas, such as the Gulf Coast, levels of up to 10 kg are recommended. Levels of 2.5 - 5 kg have been found effective in interior latex paint.

NOPCOCIDE N-96 is compatible with zinc oxide in latex paints.

NOPCOCIDE N-96 can be used with either unmodified or alkyd modified acrylic, vinyl acrylic or polyvinyl acetate latices.

If a can preservative is used in combination with **NOPCOCIDE N-96**, its compatibility with **NOPCOCIDE N-96** should be examined first. Our studies indicate that certain can preservatives appear to react with **NOPCOCIDE N-96** to produce a yellowing in the can.

Solvent-Based Exterior Paints

NOPCOCIDE N-96 can be used to aid in protecting solvent-based exterior paint films from mould growth at treatment levels of 5 - 12 kg per 1,000 litres. Again, dispersion is readily accomplished during the pigment grinding operation. As in the case of latex paints, zinc oxide can be used with **NOPCOCIDE N-96**

USE PRECAUTIONS

The following precautions should be observed in handling and using **NOPCOCIDE N-96.**

Do not get in eyes. **NOPCOCIDE N-96** is corrosive and capable of causing severe eye damage. Do not take internally. Avoid breathing dust. Avoid contact with skin or clothing.

NOPCOCIDE N-96 may produce temporary allergic side effects characterised by redness of the eyes, mild bronchial irritation and redness or rash on exposed skin areas. Persons having allergic reaction should contact a physician. Affected persons respond to treatment with antihistamines or steroid creams and/or systemic steroids.

NOPCOCIDE N-96 is toxic to fish. Do not apply directly to water. Do not contaminate water by cleaning of equipment or disposal of wastes.

Store **NOPCOCIDE N-96** in a dry place. Do not contaminate water, food or feed by storage or disposal. Open dumping is prohibited. Do not re-use empty container.

Pesticide or rinse residues that cannot be used or chemically reprocessed should be disposed of in a landfill approved for pesticides, or buried in a safe place away from water supplies. Do not contaminate water by cleaning of equipment or disposal of wastes. Containers should be disposed of in an incinerator or landfill approved for pesticide containers, or buried in a safe place. Consult federal, state or local disposal authorities for approved alternative procedures such as limited open burning.

PERSONAL PROTECTIVE EQUIPMENT

Wear goggles or eye shield when handling **NOPCOCIDE N-96** to protect eyes.

Use protective barrier cream on exposed skin areas.

Wear gloves and protective clothing when handling or using **NOPCOCIDE N-96** to minimise skin contact.

Do not inhale dust - wear a respirator if necessary.

Launder outer garments if exposed to dust or spills. Discard footwear which cannot be decontaminated.

TYPICAL PROPERTIES

Chemical	-	Tetrachloroisophthalonitrile*
Active Content, %	-	96
Empirical Formula	-	$C_8Cl_4N_2$
Molecular Weight	-	265.9
Melting Range	-	250 - 251°C
Boiling Point,°C	-	350 at 760 mm Hg
Vapour Pressure, 40°C	-	< 0.01
170.4°C	-	9.2
190.8°C	-	17.4
211.5°C	-	27.3
229.5°C	-	43.3
Hydrolysis Rate	-	Does not hydrolise in either moderate alkal or acidic aqueous media.
Physical State	-	3 - 5 micron micromilled powder
Colour	-	Grey
Odour	-	Pure - Odourless Technical - Slight pungent odour.
Stability	-	Thermally stable under normal temperature storage. Chemical stable in alkaline or aci aqueous media. Stable to ultra-vic radiation.
Corrosivity	-	Non-corrosive to metals.
Bulking Factor, litres/kg	-	0.67
Specific Gravity (H_2O = 1)	-	1.8

* U.S. Patents 3,920,353 and 3,331.735
EPA Reg. No. 50534-114-2204

FIRST AID PROCEDURES

Contact with eyes: Flush eyes with plenty of water immediately for at least 15 minutes. Seek medical attention immediately.

Contact with skin: Wash exposed areas of skin with soap and warm water after handling or using **NOPCOCIDE N-96**. Contaminated clothing including footwear should be thoroughly cleaned before re-use.

Inhalation: Remove to fresh air.

Ingestion: Do not induce vomiting. If vomiting occurs spontaneously, keep airway clear. Never give anything by mouth to an unconscious person. Get medical attention.

Allergic reactions: Contact a physician immediately for treatment with antihistamines or steroids.

CONTAINER

NOPCOCIDE N-96 is shipped in 45 kg net weight plastic lined fibre drums.

Material Safety Data Sheet

COMPANY DETAILS

DATE OF ISSUE: February, 1993

MANUFACTURER'S NAME & ADDRESS:

Henkel Corporation
300 Brookside Avenue
AMBLER, PENNSYLVANIA, USA

EMERGENCY TELEPHONE NO.:

(03) 309 5188 Henkel Australia Pty. L
83 Maffra Street
BROADMEADOWS VIC.

PRODUCT NAME: NOPCOCIDE N-96

IDENTIFICATION

OTHER NAMES:

Tetrachloroisophthalonitrile and Chlorothalonil.

USE:

Fungicide for agriculture and surface coatings.

DANGEROUS GOODS INFORMATION

DESCRIPTION: Poisonous, solid, n.o.s., harmful mixture.

U.N. NO.: 2811 **CLASS: 6.1** **HAZCHEM CODE: 2[Z]**

EMERGENCY PROCEDURE GUIDE: 6B5 **POISONS SCHEDULE: S 6**

PHYSICAL DESCRIPTION/PROPERTIES

APPEARANCE:	**White Powder**	**ODOUR:**	**Slightly pungent**
SOLUBILITY IN WATER:	**0.6 ppm**	**BOILING POINT:**	**> 350°C**
pH:	**5 - 7**	**MELTING POINT:**	**Not Determined**
FLASH POINT:	**Not Applicable**	**SPECIFIC GRAVITY:**	**1.8**
FLAMMABILITY LIMITS:	**Not Applicable**	**VAPOUR PRESSURE:**	**Not Determined**

INGREDIENTS

CHEMICAL ENTITY	CAS. NO.	PROPORTION
Tetrachloroisophthalonitrile	**1897-45-6**	**96%**
Inert Ingredients	**-**	**4%**

IDENTIFICATION

PRODUCT NAME: Busan 11-M1

UN NUMBER: None allocated
HASCHEM: None allocated
CLASS: None allocated
POISON: S6

USES

Industrial microbicide, corrosion inhibitor in paints.

PHYSICAL DESCRIPTION / PROPERTIES

Appearance	Fine white powder
Boiling Point	Not applicable
Vapour Pressure	Not applicable
Specific Gravity	3.3-3.4 g/mL, packed
Flash Point	Does not flash
Flammability Limits	Not applicable
Solubility In Water	0.3 - 0.4% in water at 25 C.
pH, neat	Not applicable
pH, 1% soln	6-7 in pH 7 water

INGREDIENTS

Chemical Name	CAS Number	% by weight
Barium Metaborate Monohydrate	13701-59-2	90 minimum
Impurities		Balance

.... 2/

HEALTH HAZARD INFORMATION

HEALTH EFFECTS

1. Effects from acute exposure

IF SWALLOWED: No data is available on human ingestion of Busan 11-M1. May be harmful. Acute oral LD50 (Rats): Male: 850 mg/kg, Female: 530 mg/kg.

EYE: Eye irritant. Effects may range from mild to severe (mechanical irritation) depending on length of exposure, concentration, and first aid measures.

SKIN: Not a primary skin irritant. Mild skin irritation may develop depending on length of exposure, concentration, and first aid measures. Acute dermal LD50 (Rabbits): greater than 2000 mg/kg.

INHALED: May cause irritation or corrosion of mucous membranes and the lungs. Exposed individuals should be monitored for respiratory distress, bronchitis, or pneumonia. Acute inhalation LC50 (Rats): greater than 3.54 mg/L (four hour study).

2. Effects from chronic exposure

The effects from chronic exposure to Busan 11-M1 have not been fully evaluated.

As with any chemical, ingestion, inhalation, and prolonged and repeated skin contact should be avoided by good occupational work practice.

FIRST AID

If poisoning occurs, contact a doctor or Poisons Information Centre.
IF SWALLOWED: INDUCE VOMITING. Rinse mouth with copious amounts of water or milk, first. Vomiting should be induced using Ipecac syrup APP. (Adult dosage = 2 Tablespoons). This should then be followed by 1 to 2 glasses of water. In cases where the individual is semi-conscious, unconscious, or convulsing, DO NOT GIVE FLUIDS BY MOUTH. Take individual to nearest medical facility.

EYES: Flush immediately with copious amounts of tap water or normal saline (minimum of 15 minutes). Take exposed individual to a health care professional, preferably an ophthalmologist, for further evaluation.

SKIN: Wash exposed area with plenty of soap and water. Repeat washing. Remove contaminated clothing and wash thoroughly before reuse. If irritation persists, consult a health care professional.

INHALATION: Move the exposed individual to fresh air - avoid becoming a casualty.

.... 3/

ADVICE TO DOCTOR

No specific antidote is known. Treat symptoms. Use of saline or osmotic cathartics is recommended. Medical consultation is available 24 hours a day. Call the Buckman office on (018) 255 124.

PRECAUTIONS FOR USE

EXPOSURE STANDARDS

No exposure standard has been allocated for this product. However, there is a recommended exposure standard for dust in general, which is 10 mg per cubic metre.

ENGINEERING CONTROLS

Use only in a well ventilated area. Maintain concentration below recommended exposure limits.

PERSONAL PROTECTION

Safety glasses with shields, and chemical impervious gloves are recommended. Nuisance dust masks are required when air levels exceed the recommended exposure limit of 10 mg per cubic metre. Body protective clothing such as long sleeves and long trousers are recommended.

FLAMMABILITY

Not flammable under conditions of use.

SAFE HANDLING INFORMATION

STORAGE AND TRANSPORT

Store in shipping container in a cool, dry, ventilated area, away from direct sunlight. Area should be constructed with suitable flooring and bunding (according to local authority requirements) designed to fully contain spills. Do not store near strong acid.

TRANSPORT:
UN No. None allocated
ACTDG Class: None allocated
Packaging Group None allocated

....4/

SPILLS AND DISPOSAL

LARGE SPILLS: Sweep up all material. Dispose of as below.

SMALL SPILLS: As for large spills.

DISPOSAL: Note Follow federal, state and local regulations governing the disposal of waste materials.
PRODUCT DISPOSAL: Product is not a hazardous waste and can be disposed of in an approved landfill.

CONTAINER DISPOSAL: Empty bags are not hazardous and may be disposed of in an approved landfill.

FIRE/EXPLOSION HAZARD

FLAMMABLE LIMITS: Not applicable.

EXTINGUISHING MEDIA: Not applicable

HAZARDOUS DECOMPOSITION PRODUCTS: Contact with strongly acidic aqueous systems can release soluble Barium salts, which are highly toxic by ingestion.

SPECIAL FIREFIGHTING PROCEDURES: Breathing apparatus, and protective gloves, are recommended.

CONTACT POINT

Technical Manager 02) 725 5811
General Manager 018) 255 124

The information on this Material Safety Data Sheet reflects the latest information and data that we have on hazards, properties and handling of this product under the recommended conditions of use. Any use of this product or method of application which is not described in the product data sheet is the responsibility of the user.

Reprinted from

Aquaculture

Aquaculture 152 (1997) 77–90

Biofouling of fish-cage netting: efficacy and problems of in situ cleaning

Stephen L. Hodson [a,*], Thomas E. Lewis [b,1], Christopher M. Burke [a]

[a] *Department of Aquaculture, University of Tasmania at Launceston, P.O. Box 1214, Launceston, Tas. 7250, Australia*

[b] *Salmon Enterprises of Tasmania Pty Ltd, P.O. Box 1, Wayatinah, Tas. 7140, Australia*

Accepted 31 December 1996

ELSEVIER

ELSEVIER

Aquaculture 152 (1997) 77–90

Aquaculture

Biofouling of fish-cage netting: efficacy and problems of in situ cleaning

Stephen L. Hodson [a,*], Thomas E. Lewis [b,1], Christopher M. Burke [a]

[a] *Department of Aquaculture, University of Tasmania at Launceston, P.O. Box 1214, Launceston, Tas. 7250, Australia*

[b] *Salmon Enterprises of Tasmania Pty Ltd, P.O. Box 1, Wayatinah, Tas. 7140, Australia*

Accepted 31 December 1996

* Corresponding author. Tel.: (+61-3) 6324 3816; fax: (+61-3) 6324 3804; e-mail: Stephen.Hodson@utas.edu.au.

[1] Present address: Department of Agricultural Science, University of Tasmania, G.P.O. Box 252–54, Hobart, Tas. 7001, Australia.

PII S0044-8486(97)00007-0

Biofouling, 1995, Vol. 9 (2), pp. 145–151
Reprints available directly from the publisher
Photocopying permitted by license only

Short Communication

IN SITU QUANTIFICATION OF FISH-CAGE FOULING BY UNDERWATER PHOTOGRAPHY AND IMAGE ANALYSIS

STEPHEN L HODSON[1], CHRISTOPHER M BURKE[1] and THOMAS E LEWIS[2,3]

[1]*Department of Aquaculture, University of Tasmania at Launceston, PO Box 1214, Launceston, Tasmania 7250, Australia*
[2]*Salmon Enterprises of Tasmania Pty Ltd, Marine Operations, PO Box 103, Dover, Tasmania 7117, Australia*
[3]*Current address: Department of Agricultural Science, University of Tasmania, GPO Box 252C, Hobart 7001, Australia*

(Received 24 May 1995; in final form 17 August 1995)